Department of Forensic Science

TOXICOLOGY PROCEDURES MANUAL

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1 INTRODUCTION

1.1 Introduction

The Section analyzes blood and other biological samples for the presence of alcohol, drugs and poisons. Types of cases analyzed include DUI/DUID (Driving under the Influence/ Driving under the Influence of Drugs), drug-facilitated sexual assaults, death investigations and non-implied consent cases (possession, manslaughter and search warrants).

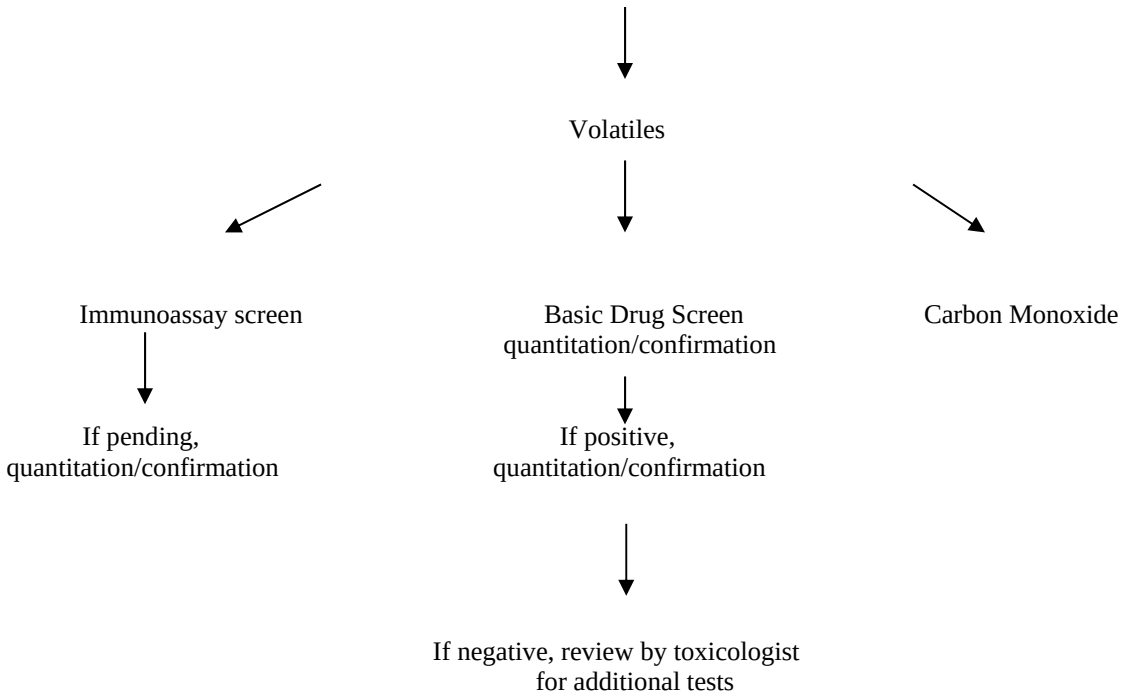
1.2 Toxicology Analytical Schemes Introduction

The toxicology laboratory in Richmond includes a regulatory alcoholic beverage laboratory supported by the Virginia Department of Alcoholic Beverage Control (ABC). This ABC lab tests beverages seized by law enforcement to determine alcohol content.

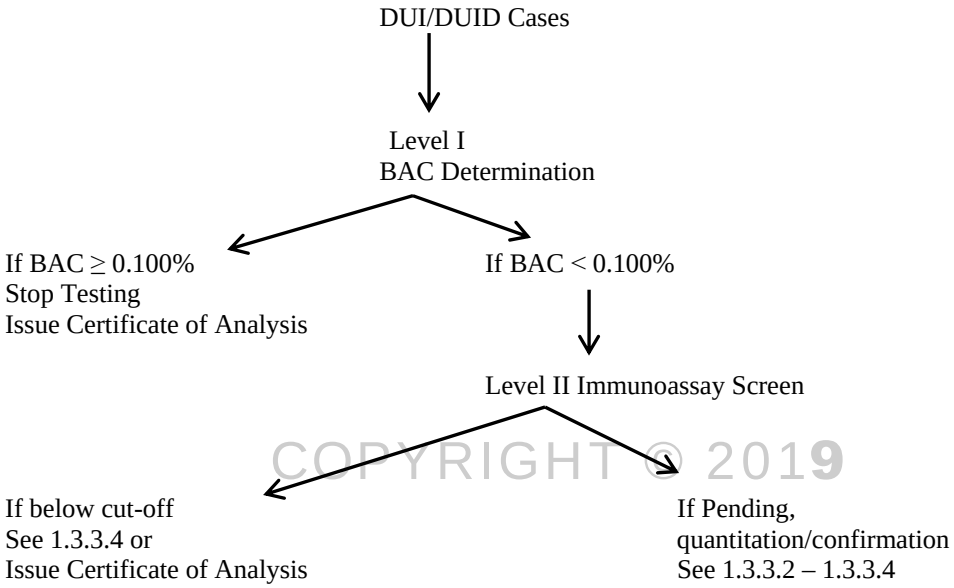
1.3 Toxicology Analytical Schemes

1.3.1 The following are general analytical schemes to be used for toxicology cases. Medical Examiner (OCME) and Toxicology – Other (TO) case testing is decided based on information on the Request for Laboratory Examination Form and case history. The cases are too diverse to have a specific analytical scheme: however, all cases follow a general analytical protocol. The DUI/DUID protocol is designed to identify alcohol and drugs that can impair driving using two levels of testing: alcohol and impairing drugs. Once potentially impairing levels of alcohol or drugs have been identified, the testing may be stopped and a Certificate of Analysis is generated. Exceptions may be necessary due to customer requests for additional testing.

1.3.2 Medical Examiner / Toxicology-Other (e.g., non-DUI/DUID cases, maiming, manslaughter, sexual assault, poisoning)



1.3.3 DUI/DUID (Implied and Non-Implied (Search Warrant) Consent) Cases



1.3.3.1 Analyze all samples for ethanol

1.3.3.1.1 If ethanol is < 0.100%, include it in the report, and go to ¶ 1.3.3.2.

1.3.3.1.2 If ethanol is ≥ 0.100%, stop; report results.

1.3.3.2 Perform Level II Immunoassay Drug Screening:

1.3.3.2.1 If any drug is tentatively present, go to ¶ 1.3.3.3.

1.3.3.3 Perform Level II Identification/Quantitation. Refer to Appendix C for stop testing concentrations. If stop testing concentrations are met on any quantitations, testing may be stopped under the guidance of a toxicologist and results may be reported.

1.3.3.4 Additional testing, including base screens, may be conducted at the discretion of a toxicologist.

2 TOXICOLOGY QUALITY GUIDELINES

2.1 Summary

The following toxicology quality guidelines apply for the analysis and subsequent accepting and reporting of toxicology results, unless otherwise specified in a specific method SOP.

2.1.1 Minor deviations and exceptions shall be authorized by the section supervisor and documented in the case file.

2.1.2 Major deviations and exceptions shall be authorized by the Program Manager and documented in the case file with an MFR.

2.1.3 When such deviations and exceptions affect the original contract, they shall be communicated to the customer and this communication shall be documented in the case file.

2.2 Guidelines for Confirming Positive Results

2.2.1. As a general matter of forensic principle, the detection of drugs and other toxins should be confirmed (whenever possible) by a second technique based on a different chemical principle.

2.2.2. If a second technique is not available, the identification must be confirmed on a different aliquot of the same specimen or in a second specimen.

2.2.3. Whenever possible, the confirmatory (second) test should be more specific and sensitive than the first test for the target analyte. Mass spectrometry is recommended as the confirmatory technique. Exceptions include analytes which are not readily analyzed by mass spectrometry such as carbon monoxide, volatile hydrocarbons (alcohols), anti-inflammatory medications and heavy metals.

2.2.4. The following are acceptable confirmatory practices in order of preference. At least one condition must be satisfied in order to identify and report a drug.

2.2.4.1 Identification of the substance class and specific identification of the substance in a different aliquot by a different chemical principle (e.g., immunoassay followed by GC-MS SIM quantitation).

2.2.4.2 Identification of the substance in more than one aliquot by different chemical principles (e.g., base screen identification of antidepressant by GC-MS followed by quantitation by GC-NPD or selective immunoassay for acetaminophen or salicylate followed by quantitation by HPLC and diode array spectral match and identification.).

2.2.4.3 Identification of the substance in different biological samples by one or more chemical principles (e.g., positive immunoassay for opiates in blood, confirmation of morphine in vitreous humor).

2.2.4.4 Identification of the substance in one biological sample using two separate aliquots and one chemical principle (e.g., ethanol analysis by headspace GC-FID).

2.2.4.5 Identification of an incidental substance by mass spectrometry in one aliquot and case history verifies the identification (e.g., lidocaine confirmed in base screen by mass spectrometry in postmortem case involving medical resuscitation).

2.3 Guidelines for Batch Analysis

2.3.1. In order to maximize efficiency within the toxicology laboratory, it is common to group specimens into batches. The following are general guidelines for batch analysis, unless otherwise specified in a method SOP.

2.3.2. The transfer, handling and aliquoting of specimens is documented with the analyst's signature and date on the batch summary worksheet. Each batch worksheet also contains the unique FS lab number where the original raw data (including chromatograms of calibrators/controls, calibration curves, instrument sequence lists, etc.) shall be stored. The analysis start and end dates are defined on the Toxicology Batch Worksheet (or equivalent document). The start of the analysis date is the analyst custody date at the bottom of the form. The analysis completion date is the date of data review noted on the Toxicology Batch Worksheet (lower right).

2.3.3. Each batch analysis must contain a sufficient number of controls to monitor the performance of the assay; however, the total number of controls will depend on the size of the batch and the nature of the test.

2.3.3.1 For qualitative screening tests such as rapid presumptive tests and basic drug screens, the batch should contain the appropriate batch summary worksheet and a minimum of one negative control and one positive control/reference standard.

2.3.3.2 For quantitative analysis, each batch of specimens should include 10% controls, including at a minimum, one positive and one negative control.

2.3.3.3 A solvent blank (double blank), a matrix blank (blank blood or matrix with no standards or internal standards), or negative control (blank blood or matrix with internal standard) shall be run after the highest spiked calibrator in every batch to monitor for method carryover.

2.3.4. For batch analyses that utilize instrument autosamplers:

2.3.4.1 Case samples shall be bracketed by an acceptable calibrator or control.

2.3.4.2 The identity of each vial in the autosampler must be verified by an independent analyst with the autosampler sequence list and/or worksheet. This vial verification is documented with initials and date on the sequence table.

2.3.5. If a batch analysis fails to meet the QA/QC acceptance criteria, at minimum, a copy of the batch worksheet containing documentation of specimen aliquots and the reason for the batch failure shall be included in each case file.

2.3.6. For quality assurance purposes, each batch analysis must be reviewed by an independent analyst and this review is documented with a signature and date on the batch worksheet. The batch analysis review process includes a review of the following items:

2.3.6.1 Batch summary worksheet with chain of custody and raw data file location.

2.3.6.2 Documentation of vial verification

2.3.6.3 Autosampler sequence list

2.3.6.4 Calibrator chromatograms

2.3.6.5 Control chromatograms

2.3.6.6 Calibration tables and curves (for quantitative assays)

2.3.6.7 QA/QC summary sheets and UoM worksheets if applicable. These sheets must be reviewed for content, accuracy and data transfers. This review is documented with signature and date on the QA/QC summary sheet. For LCMSMS analysis, the QA/QC summary sheet is equivalent to the LCMSMS summary report. For GC-MS analyses that utilize MassHunter data analysis, the Quantitative Summary Report (or equivalently titled document) is

equivalent to the QA/QC summary sheet. The review of the LCMSMS or GC-MS summary report is noted by the reviewer's signature and date on the batch worksheet.

2.3.6.8 Instrumental parameters, except Immunoassay and UV Spectrophotometry

2.3.6.9 All case sample chromatograms.

2.3.7. Once the batch summary review process is complete, a photocopy of the batch worksheet, QA/QC summary sheet for positive results, and UoM worksheets (if applicable) are placed in each case file with the case sample chromatograms.

2.4 Quality Assurance and Quality Control

2.4.1. Definitions

2.4.1.1 Linear range: For most chromatographic assays, the Limit of Quantitation (LOQ) and Upper Limit of Quantitation (ULOQ) are administratively defined in terms of the concentration of the lowest and highest calibrator used in the calibration response curve.

2.4.1.2 Limit of detection (LOD): The LOD is the lowest concentration where an analyte can be confirmed as present, but not necessarily quantitated accurately.

2.4.1.3 LOQ: The LOQ is the lowest calibrator concentration included in the calibration response curve. To include the LOQ in calibration response curve, the LOQ must be at least three times greater than the negative control response, must have a greater response than the LOD spike, must have acceptable ion ratios and must back calculate within 30% of target concentration. If the LOQ does not meet these criteria, then the new LOQ is the lowest calibrator that satisfies these criteria or the assay is repeated.

2.4.1.4 ULOQ: The ULOQ is the highest calibrator concentration included in the calibration response curve with acceptable ion ratios and whose back-calculated concentration falls within 20% of target concentration.

2.4.2. Qualitative Assays

Analyze a minimum of one negative and at least one positive control/reference standard along with unknowns in each chromatographic and rapid presumptive screening test.

2.4.3. Quantitative Assays

2.4.3.1 Calibrators: Solutions that are prepared from a certified reference material, where possible, that are used to calibrate an assay.

2.4.3.2 Prepare a minimum of three different calibrators in each quantitative assay or as specified in the method SOP. The concentration of the calibrators should be such that they bracket the anticipated concentration of the unknown specimen(s).

2.4.3.3 Prepare a response curve of area (height) of analyte to area (height) of internal standard ratio versus calibrator concentration. Calculate the analyte concentration by interpolation of the linear plot. It is acknowledged that some assays are inherently non-linear (e.g., LCMSMS) and the use of quadratic models may be necessary and appropriate, and should be verified using low, medium and high controls. The response curve and determined unknown specimen concentration(s) are generated by the instrument software.

2.4.3.4 Calculate the coefficient of determination (r^2) for the curve. For most applications, an r^2 of greater than 0.985 is acceptable (unless otherwise indicated in a specific method SOP).

2.4.3.5 Evaluate the curve by back-calculating calibrator concentrations against the curve. Values of ± 30% from the target calibrator concentration are acceptable for the lowest calibrator. All other calibrators must fall within 20% of target calibrator concentration unless otherwise indicated in a specific method SOP.

2.4.3.6 Calibration curve data point inclusion guidelines:

- At least 3 calibrators must be included in the calibration curve.
- All positive controls must be greater than the LOQ and less than the ULOQ.
- No more than 3 calibrators between the LOQ and ULOQ may be removed from the calibration curve. Note: the reason for removing the calibrator shall be clearly indicated through notations from the examiner or by the computer software on the data.

2.4.3.7 If the lowest calibrators are removed from the curve, this change in LOQ may require a repeat analysis of case specimens below the new LOQ. Alternatively, the change in LOQ should be communicated to the customer and documented with an MFR or the change can be communicated on the CoA by reporting "none detected at (new LOQ)" or "Present, less than (new LOQ)."

2.4.3.8 If more than 3 calibrators are removed from the curve, this exception and supporting justification shall be authorized by the Supervising Toxicologist and documented in the case file with an MFR.

2.4.4. Internal standards: Internal standards are required for chromatographic assays.

2.4.4.1 It is preferable to use an internal standard with similar extraction, derivatization, and chromatographic properties to the analyte(s) of interest.

2.4.4.2 The use of stable isotope internal standards for selected ion monitoring GC-MS is encouraged but not required since well-chosen non-deuterated internal standards may give similar performance.

2.4.4.3 The volume of internal standard solution prepared may be modified from the volume indicated in the methods herein, however the concentration of the internal standard must remain the same as directed.

2.4.4.4 The internal standard recovery as measured by peak area/height or ion abundance should be monitored for calibrators, controls and case specimens.

For methods utilizing the LCMSMS instrument, the signal to noise (S/N) calculated by the instrument software may be monitored as well.

2.4.4.5 To maintain method reliability, the internal standard (IS) recovery of the samples should be similar to that of the calibrators and controls. During data review, the examiner and reviewer should be mindful of these recoveries and remediate accordingly if it appears that the IS recovery of the sample falls below approximately half of the IS recovery of the calibrators and controls. Remediation actions, if appropriate, include the following:

- For methods utilizing the MassHunter software, note the S/N calculated by the instrument software. If the S/N of the quantitation transition/ion is greater than 10:1, the data may be accepted. This acceptance will be recorded on the data. If the S/N is less than 10:1, continue with remediation actions.
- Reextract and reanalyze the specimen.

- Reinject the affected case sample to determine whether or not poor recovery was due to a poor injection. Document the reinjection on the chromatogram with "reinjection."

- Upon reinjection, if the internal standard recovery still falls below approximately 50% of average internal standard recovery:

  - The case specimen may be reextracted to obtain a quantitative value.

  - The case specimen may be reanalyzed using a different internal standard. This major deviation and exception shall be authorized by the Program Manager and documented in the case file with an MFR.

  - The drug may be reported as "present" if it has been confirmed. The reason for reporting "present" shall be documented in the case file and, if appropriate, communicated to the customer (see ¶ 2.6.1.9).

  - The case specimen may be reported as "unsuitable for" the particular "analysis." This exception shall be authorized by the Toxicology Supervisor or group supervisor, documented in the case file with an MFR, and communicated to the customer.

2.4.5. Controls: Controls in quantitative assays may be purchased or prepared in-house. In-house prepared controls should be prepared from a different manufacturer or different lot of standard material than used in calibrators. If this is not possible, controls should be prepared from a different weighing or dilution from the calibrators, preferably by another analyst. Results from quantitative quality control materials are recorded in control charts to readily detect trends such as deterioration of reagents, calibrators and controls.

2.4.5.1 Analyze a negative control and at least one positive control with each quantitative procedure.

2.4.5.2 In-house methanolic controls fortified in matrix:

- Positive control range is ± 20% from the target concentration.

- All staff are responsible for entering new values into the statewide control charts, if applicable.

- The DTS Research Analyst or designee should review the statewide control charts each month to ensure consistency between laboratories and communicate results with Supervising Toxicologists as necessary.

2.4.5.3 Statewide methanolic controls fortified in matrix:

- Positive control range is ± 20% from the target concentration.

- The control is verified with six replicates against calibrators prepared from another source before being placed in service.

- If acceptable, the DTS Research Analyst or designee will put the lot into service and create the control chart on the shared drive. The DTS Research Analyst or designee will notify toxicology personnel that the new control is in service.

- All staff are responsible for entering new values into the statewide control charts.

- The DTS Research Analyst or designee should review the statewide control charts each month to ensure consistency between laboratories and communicate results with Supervising Toxicologists as necessary.

2.4.5.4 External purchased controls (i.e., UTAK or equivalent):

- Positive control range is ± 20% from the mean concentration established by statewide analysis.
- Three replicates from each laboratory shall be used to set the DFS mean, unless otherwise specified. For controls used in the "Carboxyhemoglobin Quantitation and Confirmation" analysis (¶ 10)), the DFS mean is established from twelve replicates from at least three laboratories. For controls used in the "Ethanol Content of Alcoholic Beverages by Headspace GC" analysis (¶ 24), the DFS mean is established from three replicates from four batches for a total of twelve values.
- The DTS Research Analyst or designee will put the lot into service and create the control chart on the shared drive.
- All staff are responsible for entering new values into the statewide control charts.
- The DTS Research Analyst or designee should review the statewide control charts each month to ensure consistency between laboratories and communicate results with Supervising Toxicologists as necessary.

2.4.5.5 The target analyte concentrations of positive controls must be between the lowest and highest calibrator. For assays using one positive control, it is preferred that the control concentration is approximately midrange of the curve. If multiple positive controls are used, control concentrations preferably should monitor the overall performance of the assay (low, medium and high controls).

2.4.5.6 Each quantitative batch analysis should contain 10% controls. When multiple positive controls are run within a batch, 2 out of 3 positive controls (or 67%) must fall within the acceptance criteria for in-house methanolic controls, state wide methanolic controls, and external purchased controls used within the batch for an acceptable quantitative analysis. If controls do not meet these acceptance criterions, all positive case samples should be repeated. However, qualitative results may be reported when reanalysis is not possible or practical.

2.4.5.7 In addition to the positive controls, a LOD spike shall be run at half the concentration of the lowest calibrator. The LOD spike is not part of the calibration curve, but assists in determining the LOD for the batch.

2.4.5.8 The negative control must indicate that the analyte of interest is below the LOD spike of the assay. In general, the negative control should not contain enough analyte of interest to be confirmed.

2.4.6. Pipette Utilization

Toxicology personnel shall not pipet less than 10 µL for calibrators.

2.4.7. The Department's laboratory facilities provide sufficient environmental conditions to conduct all tests listed in this Procedures Manual with no further consideration required.

2.5 Chromatographic and Mass Spectral Quality Control

2.5.1. Chromatographic quality control.

Some toxicology casework may contain multiple drugs or co-eluting decomposition products that may prohibit adherence to some of the following chromatography guidelines. Exceptions shall be documented in case notes or on chromatograms.

2.5.1.1 Retention Time: Retention times for both analyte and internal standard must be within ± 2.0% of the retention time obtained from the average of the calibrators. Larger deviations (10%) are acceptable for assays based on HPLC, particularly with procedures in which the mobile phase programming is non-isocratic.

2.5.1.2 Peak Resolution: To the greatest extent possible, chromatographic peaks used for quantitative analyses should be resolved from interfering peaks such that the signal height of the valley between adjacent peaks is no greater than approximately 10% of the peak height of interest.

2.5.1.3 Peak Width: Measured at the base of the peak, chromatographic peaks of interest should be at least approximately 10% of SIM window width to permit diagnostic review.

2.5.1.4 Peak Symmetry: Peak shape should be reasonably symmetrical and return to at least 10% of peak height.

2.5.2. Mass spectral quality control.

2.5.2.1 Full scan mass spectral identification: No rigid mass spectral probability based match criteria are defined to identify a drug. Flexibility is given to the experienced interpreter because rigid criteria can lead to misidentification as well as under-identification. The experienced interpreter will base identification on a number of factors, such as retention time, unique ions, ion abundance, S/N and literature references as well as probability based matching scores.

- Whenever possible, the identification of a drug should be based on a spectral library match or comparison to an actual standard reference material. This standard reference material is required to verify the mass spectrum and retention time.

- For qualitative identification, the analyte should be compared to a positive control or calibrator containing standard reference material (for mass spectrum and retention time verification) and a negative control. The case sample analyte peak should be well resolved, the retention time should match the standard and the mass spectrum should contain all of the major and diagnostic ions unique to the analyte. Missing ions or the presence of additional ions in the unknown sample is indicative of a weak signal, background noise or co-eluting substances.

- Selected ion monitoring (SIM) identification: When SIM is used for identification of an analyte, whether as part of a quantitative procedure or not, retention time match is required and the use of at least two analyte qualifying ions and one internal standard qualifying ion is preferred.

2.5.2.2 Multiple reaction monitoring (MRM) identification: In tandem mass spectrometry methods, when MRM mode is used for identification of an analyte, as part of a quantitative procedure or not, retention time match and the use of two analyte transitions and one internal standard transition is required.

2.5.2.3 For SIM and MRM analyses, acceptance criteria for ion and transition ratios are ± 20% or 2SD relative to the average ratio from all calibrators used in the calibration response curve. However, it is recognized that some ion ratios are concentration dependent and that comparison to a calibrator of similar concentration may be necessary rather than the average ratio for the curve. This exception shall be documented in case notes or batch summary raw data file.

2.5.2.4 When developing new methods for quantitation and identification, isotope or adduct ions should be avoided as qualifier ions.

2.5.3. HPLC/DAD quality control and spectral matching

When using diode array detection and UV spectra library matching for confirmation and identification (salicylate and acetaminophen), the following criteria should be met:

2.5.3.1 The analyte relative retention time should fall within 10% of target.

2.5.3.2 Similarity index or spectral match should be greater than 995.

2.5.3.3 The identification of the analyte should correlate with the Immunoassay results.

2.5.4. Reinjection criteria and documentation

2.5.4.1 Occasionally, there may be situations in which calibrators, controls and case samples need to be reinjected on the chromatographic instrument. Common reasons for reinjection include the following:

- Poor analyte or internal standard recovery. The vial is reinjected to determine whether poor recovery is due to the injection or extraction.

- Sample overload. Add solvent to the vial and reinject or reduce injection volume and reinject.

- Poor chromatography or interference. Add or change solvent and reinject.

- Carryover. Carryover may occur due to the use of automated chromatographic injection systems or due to the extreme range of drug concentrations detected in toxicological specimens. Regardless of cause, extreme caution is warranted when carryover is detected and requires supervisory toxicologist or group supervisor notification, guidance and review of the analytical results. Appropriate actions may include reinjection with solvent blanks between specimens or reanalysis of some or all of the specimens. The supervisory toxicologist or group supervisor shall document their review of the carryover, root cause analysis and the appropriate response on an MFR to be included with batch summary raw data file and affected individual case files.

    - Any sample run directly after a sample which has an analyte concentration greater than the highest calibrator shall be reinjected to assess carryover. If a solvent blank is run after a sample which has an analyte concentration greater than the ULOQ and no carryover is detected, no reinjection analysis is necessary. Also, if a sample is run after a sample which has an analyte concentration greater than the ULOQ and is negative for the analyte in question (e.g., Sample 1 – morphine present greater than 0.80 mg/L; Sample 2 – morphine ND, oxycodone 0.14 mg/L), no reinjection analysis or MFR is necessary.

2.5.4.2 Documentation of reinjection.

- Document on the original chromatogram that the original injection was unacceptable using language such as “Not used due to (reason for reinjection).”

- Document on the reinjected chromatogram, “reinjection.”

2.6 Criteria for Reporting Toxicology Case Results

2.6.1. Drug reporting guidelines: Report drug concentrations in mg/L on the certificate unless otherwise stated in a specific method SOP.

2.6.1.1 Results shall be reported to two (2) significant figures with the exception of ethanol, acetone, isopropanol, and methanol which are reported to three (3) decimal places. Results shall be rounded using conventional rounding rules (see Quality Manual ¶ 5.4.6.3).

The expanded Uncertainty of Measurement shall be rounded using conventional rounding rules and reported (see ¶ 5.4). The reported measurement uncertainty shall be rounded to the same level of significance (i.e., decimal places) as the reported concentration.

2.6.1.2 For samples analyzed more than once, replicates must agree within ± 20% of the mean except for alcohols (see ¶ 7.9.8). Report the average of the replicates.

2.6.1.3 If an assay is repeated more than once, an exception to exclude one replicate from the mean ± 20% range may be made if that value causes the mean and ± 20% range to be unacceptable for all replicates. Document the exception on an MFR in the case file.

2.6.1.4 If multiple dilutions are analyzed, report the least dilute sample that falls within the quantitation range of the assay.

2.6.1.5 If biological fluids or tissues are diluted prior to the analysis, all digits will be carried through the calculation and the final result will be rounded following conventional rounding rules.

2.6.1.6 Urine results are typically reported as “present” only since the concentration provides little interpretive value.

2.6.1.7 Acquire data with at least one additional significant figure than what is reported. For drugs with low concentrations (THC and fentanyl) it may be advantageous to collect data in µg/L or ng/mL and then convert to mg/L for reporting.

2.6.1.8 A drug may be reported as “Present” for a number of reasons including specific reporting guidelines in a method SOP (e.g., 6-acetylmorphine), quantitative procedures were not performed or available or a quantitative procedure was performed but acceptance criteria were not satisfied and reanalysis is not possible or practical.

- To report a drug as “Present,” it must be confirmed (see ¶ 2.2).
- Confirmed drugs not specifically requested for testing by the customer or listed in the case history may be reported “Present” without notification of the customer.
- Confirmed drugs requested by the customer or listed in the case history require prior notification to the customer to be reported “Present” and shall be appropriately documented in the case file.
- “Present” may be used to report the presence of drugs in different tissues for consistency and support of analytical findings (e.g., report a case with postmortem methadone findings of blood 1.3 mg/L, liver 2 mg/kg and urine “present”).

2.6.1.9 Report drug as “Present, Less than __” if the drug has been confirmed but less than the LOQ and greater than or equal to the LOD of the batch. Report as “Present, Less than (LOQ concentration).” If a dilution factor was used in the analysis, multiply the dilution factor by LOQ to use for the reported LOQ. Exceptions may be approved by the supervisor, group supervisor, or toxicologist on a case-by-case basis.

2.6.1.10 Report drug as “Present, Greater than __” if the confirmed drug concentration was greater than the ULOQ of the assay and reanalysis is not possible or practical. Report as “Present, Greater Than (ULOQ concentration).” If a dilution factor was used in the analysis, multiply the dilution factor by ULOQ to use for the reported ULOQ.

2.6.1.11 Report as “None Detected at __.” A quantitative analysis was attempted and the drug did not satisfy confirmation criteria or was below the LOQ or LOD. The reported concentration is the LOQ or LOD of the assay. If a dilution factor was used in the analysis, multiply the dilution factor by LOQ to use for the reported LOQ.This may also be reported as “None Detected.”

2.7 Report Wording

2.7.1. Only positive, confirmed results will be reported (see 2.6.1) with the exceptions noted in ¶¶ 2.6.1.8 – 2.6.1.11 for reporting “Present, Less than_,” “Present Greater than_,” and ”None Detected at_,”. Additional reporting language may be used to report uncommon scenarios (e.g., “Quantity insufficient for further analysis”).

1. For DUI/DUID (Implied Consent and Search Warrant) cases that have negative results, the examiner shall use the following language:

   “Blood Alcohol none detected
   No drugs and/or drug classes were detected.”

2. For OCME and TO cases with negative results, the examiner shall use the following language:

   “Ethanol none detected”

   The above statement should be followed by:

   “No drugs and/or drug classes were detected.”

   If no alcohol testing was conducted, this statement would be the only language used for negative results.

The following language may be added after the results for each item when there is no other confirmatory testing completed or the screen results are negative for all other analytes. Choose one of the following statements:

3. “No other drugs and/or drug classes were confirmed.” – This should be used in the scenario where there may be indications of drug presence however it was not confirmed.

4. “No other drugs and/or drug classes were detected.” – This should be used in the scenario where there are positive, confirmed results and all other screening shows negative results.

5. “No drugs and/or drug classes were confirmed.” – This should be used in the scenario where screening is completed in one item and confirmed in another item.

2.7.2. The following language shall be used with every case to report the drugs and/or drug classes screened for in each item. If an item had only gone through confirmatory testing when screening was completed in a different sample (e.g., TX1 (blood) screened positive for opiates, TX1 and TX2 (vitreous) confirmation of opiates) there is no need for this list. This list should encompass all screening assays and assays that do not have immunoassay or other screening procedures. This list should be populated so that it is readily interpreted by the customer and demonstrates the screening assay(s) utilized.

1. DUI/DUID: “The specimen was screened for the following drugs and/or drug classes:” (Note: this wording may be used for driving related Toxicology Other cases)

2. OCME and Toxicology Other: "Item X was screened for the following drugs and/or drug classes:"
   - The item identifier (Item X) in the above mentioned language shall be modified to reflect the item associated with the screened list.

3. After the language indicated above, the screened list (list of drugs and/or drug classes that were screened for) shall be populated (see ¶ 8.8.5 for an example of ELISA assay wording).

   - The following table lists the analyses and their associated language for the screened list, it is not inclusive of all possible assays:

Assay	Associated Language
Basic Drug Screen (Ch. 9)	alkaline-extractable drugs
Acidic/Neutral Drug Screen (Ch. 9)	acidic/neutral drugs
Carboxyhemoglobin (Ch. 10, 11)	carboxyhemoglobin
GHB (Ch. 17)	gamma-hydroxybutyrate/lactone
Anti-epileptic Drugs (Ch. 30)	anti-epileptic drugs
Heavy Metals by Reinsch Test (Ch. 23)	antimony, arsenic, bismuth and mercury

   - For the novel psychoactive substances (Ch 30), the screening shall be reported in the following format:

     Novel psychoactive substances – (panel)
     Novel psychoactive substances – (panel, panel, panel)
     Novel psychoactive substances – all panels

     The "(panel)" or "(panel, panel, panel)" shall be replaced by the panel listed within the procedure (Ch 30).

4. At the end of the completed screened list, the list will end with a period.

2.7.3. Reporting of ethanol content for alcoholic beverages is described in ¶ 24.

2.7.4. The examiner shall report the method utilized for the quantitation and/or confirmation (preference to quantitation) of the reported compounds (refer to Appendix E for worksheet abbreviations).

2.7.5. If any of the methods in the case were performed via instrument support at a laboratory other than the originating laboratory, the examiner shall indicate this on the report before the "Supporting examination…" statement with the following language:

> "The [insert method title] was performed at the Department of Forensic Science [insert laboratory and address here]."

2.7.6. The examiner shall include the date range of testing to encompass the start date (date of custody of first analysis) and the end date (date of examination) in the following format:

> "Date(s) of Testing: [insert start date] – [insert end date]"

2.7.7. The examiner shall add the following language to the end of the report:

> "Supporting examintation documentation is maintained in the case file. The above-listed methods are the respective quantitation and/or confirmation methods in place at the time of analysis. Current methods can be found in the Toxicology Procedures Manual which can be found at www.dfs.virginia.gov/documentation-publications/manuals/. Measurement uncertainty is reported at a 95.45% level of confidence for all quantitative toxicological analyses except blood alcohol which is reported at a 99.73% level of confidence."

For ABC reports, the language at the end of the report shall be:

"Supporting examination documentation is maintained in the case file. The above-listed methods are the respective quantitation and/or confirmation methods in place at the time of analysis. Current methods can be found in the Toxicology Procedures Manual which can be found at www.dfs.virginia.gov/documentation-publications/manuals/. Measurement uncertainty for alcoholic beverages is reported at a 95.45% level of confidence."

Note: If no uncertainty values are reported, the last sentence of the above paragraph may be removed.

2.7.8. On DUI, DUID, SW-DUI, and SW-DUID case types, if two vials of blood are received and only one vial of blood analyzed, the examiner shall add the following:

"A second vial of the whole blood sample was received and was not opened or analyzed."

2.8 Guidelines for Method Validation and Verification

2.8.1. Qualitative Method Validation

2.8.1.1 When a compound is to be added to an existing method for qualitative identification purposes, a written validation plan (MFR) shall be proposed to the Section Supervisor, Toxicology Program Manager, and Research Analyst. This plan should address the following criteria (exceptions may be approved by the Toxicology Program Manager):

2.8.1.1.1 List the analyte of interest, methodology, internal standards, and any expected concentrations of analytes or internal standards.

2.8.1.1.2 The analyte should be fortified at a high concentration* and extracted and injected by itself in a minimum of three matrix samples (screened negative for analyte of interest). This is done to evaluate that the analyte does not interfere with other compound signals and to establish the correct retention times and ions/transitions to be used. Where applicable, instrumental optimization software can be utilized to determine ions/transitions. Otherwise, a literature review can aid in the identification of ions/transitions.

*High concentration is dependent upon the compound(s) of interest. This should be based upon knowledge of typical concentrations, expected concentrations, or instrumental limits.

2.8.1.1.3 The internal standard that is used for the method should also be fortified at the working concentration and extracted and injected by itself in a minimum of three matrix samples. This should be used to evaluate if the internal standard can contribute to the analyte of interest's signals.'

2.8.1.1.4 A minimum of ten matrix blank (unique and screened negative, if possible) samples should be extracted and injected without analytes or internal standards. The ten may be a mixture of matrix types (e.g., blood, urine, liver). This is used to evaluate if there are endogenous contributions to the analyte signals.

2.8.1.1.5 A minimum of three matrix samples should be fortified with commonly encountered drugs, metabolites, and other structurally similar compounds at high concentrations. This should be used to evaluate interferences from commonly encountered drugs.

2.8.1.1.6 If possible/feasible, the limit of detection (LOD) of the method should be established for the analyte. Multiple samples of the analyte should be prepared in descending concentrations at expected low level values. These will be evaluated such that the lowest concentration to meet the following criteria is considered the limit of detection for the validation (Note: an administrative level may be chosen for practical purposes that is higher (not lower) than the validation limit of detection). Once the limit of detection is established, triplicate determinations of fortified samples from at least three different matrix sources should be assessed.

- Retention Time: ± 5% (for LCMSMS) or ± 2% (for GC or GCMS)
- Qualifier/Ion Ratio (if applicable): ± 20%
- Signal-to-Noise (if applicable): ≥ 3

2.8.1.1.7 Carryover should be evaluated with matrix blanks injected immediately following progressively higher concentrations of analyte that has been fortified and extracted from matrix samples. The highest concentration that is free from carryover should be confirmed with a minimum of triplicate analyses. Regardless of whether carryover is evaluated during the validation, a plan to evaluate for carryover within a batch analysis should be established (refer to ¶ 2.5.4.1).

2.8.1.1.8 Where applicable, ionization suppression/enhancement should be addressed with neat standards and post-extraction fortified samples. Two different sets of samples at two different concentrations shall be prepared and their peak areas compared between sets. Neat samples should be injected a minimum of six times each. Post-extraction fortified samples should be analyzed with a minimum of ten matrix sources at two concentrations. Each matrix should be analyzed in triplicate over the two concentrations. If significant suppression/enhancement is present, an evaluation of the effect on the limit of detection shall be completed.

2.8.1.2 Upon approval of the Validation Plan by the Toxicology Program Manager, the validation will be conducted. During the validation, the data shall undergo technical review by another analyst.

2.8.1.3 Upon completion of the validation, a Validation Summary will be generated that summarizes the validation and asks for approval by the Toxicology Program Manager to use the method for the qualitative identification of the analyte(s) of interest. The Validation Summary will be accompanied by the supporting data and will specify the method utilized. If there are multiple options for extraction in a method, the summary will explicitly state which option is utilized.

2.8.1.4 Once the Validation Summary is approved, an MFR will be generated requesting the approval to use the method. Upon approval of the MFR, the method is then able to be used in any Toxicology section following a verification of the method.

2.8.2 Qualitative Method Verification

When a laboratory, other than the laboratory where the validation took place, wants to utilize this method it will need to be verified to be fit for use. This verification will consist of a batch analysis of the analyte (w/ IS), a blank, an LOD (if validated) or low control spike, and a double blank (no analyte or IS) in the matrix in which the validation was completed. Upon verification, the section supervisor will send an MFR indicating that it is fit for use and seeking approval to use this method. This verification (data and MFR) will be retained within the laboratory completing the verification (either in hardcopy or electronic format). Note: for efficiency, the above-listed standards for verification can be combined into a casework batch analysis which shall undergo all review processes.

2.8.3 Quantitative Method Validation

2.8.3.1 When a compound or compounds are to be added to an existing method for quantitative purposes or a new quantitative method is to be validated, a written validation plan (MFR) shall be proposed to the Toxicology Program Manager for approval after review by the TRT (or designees). This plan should address the following areas and appropriate acceptance criteria, if applicable (Note: criteria may be methodology-dependent, exceptions may be approved by the Toxicology Program Manager):

- Accuracy and Precision
- Sensitivity
- Calibration Model
- Ion Suppression/Enhancement
- Recovery
- Carryover
- Interferences (endogenous, internal standard, commonly encountered analytes)
- Dilution Integrity
- Stability
- Robustness

2.8.3.2 Upon completion of the validation plan, a validation summary document shall be written to summarize the data from the validation plan. The validation summary shall be proposed to the Toxicology Program Manager and Research Analyst for review. The validation summary will also be reviewed by the TRT (or designees).

2.8.3.3 A binder of the finalized validation shall be created to contain the method development documents, approved validation plan, approved validation summary, draft SOP, batch worksheets, and any other documents association with the validation. The binder will then be sent to the Toxicology Program Manager.

2.8.4 Quantitative Method Verification

2.8.4.1 Upon approval of quantitative method validations, a method verification is required before the method can be used in the laboratory. If a method is validated in one laboratory by toxicology staff, it does not need further verification.

2.8.4.2 The verification, at a minimum, should include the calibration curve, an LOD spike, a blank, a double blank, and controls.

3 SAMPLING PROCEDURE

3.1 Sampling evidence is critical in toxicological analysis. One must be sure that what is sampled is truly representative of the total sample submitted. The analyst must take into consideration the homogeneity (or lack thereof) among submitted biological specimens. In order to perform a toxicological analysis, a representative sample shall be removed from the biological specimen.

3.2 Biological fluids should be placed on a rocker or inverted several times to ensure sample homogeneity prior to removal of an aliquot for analysis.

3.2.1 Biological fluids may be diluted prior to analysis for a number of reasons (e.g., small sample volume or analyte concentration is greater than ULOQ).

3.2.2 For screening purposes, no less than one half of the SOP required specimen volume should be sampled.

3.2.3 For quantitations and confirmations that require dilution due to the analyte concentration exceeding the ULOQ, a minimum of 100 µL of fluid should be diluted as necessary in an appropriate blank matrix such as blank blood or water (for vitreous, urine, tissue homogenates) or as otherwise indicated in a specific method SOP.

3.3 Tissues (liver, brain, spleen) are generally considered to be homogeneous throughout the tissue: therefore, a portion of the tissue may be sampled and analyzed.

3.3.1 Weigh approximately 1 gram tissue. Add appropriate volume of water to obtain final dilution (e.g., add 4 mL water for a final dilution of 1: 4 or 1/5). Homogenize sample in a homogenizer or blender.

3.3.2 Analyze 1-2 ml of tissue homogenate (or as described in specific method SOP).

3.3.3 Multiply the analyte concentration by dilution factor and report tissue concentration in mg/Kg.

3.3.4 Tissue fluid is generally considered to be homogenous and may also be analyzed. If tissue fluid is analyzed instead of actual tissue, this should be documented in the case notes and reported as such on the Certificate of Analysis.

3.4 Gastric contents may contain pills, pill fragments and/or partially digested food material and therefore are NOT homogeneous samples.

3.4.1 Prior to the analysis of gastric contents, weigh the total gastric contents. Record total weight.

3.4.2 Homogenize the gastric contents prior to sampling to ensure homogeneity.

3.4.3 Weigh or pipet (1-2 gm or mL) of the gastric homogenate and dilute with water to achieve the desired dilution factor.

3.4.4 Analyze the diluted gastric homogenate and multiply the analyte concentration by the dilution factor.

3.4.5 Multiply the analyte concentration by the total gastric content weight and report gastric contents results as total mg analyte per total (gm) gastric content submitted.

4 EVIDENCE HANDLING AND STORAGE

4.1 Evidence Submission

The proper selection, collection, submission and storage of biological specimens for toxicological analysis are important if the analytical results are to be accurate and their subsequent interpretations scientifically sound.

4.1.1 A minimum of 10 mL of blood, serum or plasma should be submitted for non-implied consent cases requiring comprehensive toxicological analyses. If less than 10 mL sample is submitted, the analyses shall be prioritized in order to maximize the value of the toxicological analyses. If less than 10 mL of sample is submitted, DFS may not be able to complete all of the requested examinations.

4.1.2 Blood samples should be collected in gray top tubes (or equivalent) containing potassium oxalate and sodium fluoride to preserve the samples. DFS provides gray top tubes and/or blood vials containing these preservatives to the customers (DUI kits and OCME blood collection tubes).

4.1.3 It is recognized that hospital or clinical specimens collected pursuant to medical treatment may be collected in blood vials with or without preservatives. Such exceptions shall be noted in the case file with an appropriate description of the evidence (e.g., purple top, red top, green top, yellow top SST, serum, plasma etc).

4.1.3.1 If the hospital or clinical specimens differ in the collection times and the differences could affect the interpretation of the results, then the times should be recorded in the case file and reported on the Certificate of Analysis.

4.1.3.2 In general, the specimens with the earliest collection times should be analyzed whenever possible.

4.1.4 Postmortem samples should be labeled with type (e.g., blood, bile, urine, liver) and location of blood sample collection (e.g., iliac, heart, subclavian). Failure to label postmortem evidence appropriately limits the value and interpretation of toxicological results.

4.2 Evidence Receipt

At the time of receipt, the specimen label information should be inspected and compared to RFLE (if available) to verify that the information matches. Any discrepancies should be documented on a toxicology worksheet.

4.2.1 A DUI or toxicology worksheet is prepared for each case file.

4.2.2 The DUI or toxicology worksheets should document unique specimen identifiers and the evidence should be labeled accordingly.

4.2.2.1 For postmortem cases, a toxicology worksheet is prepared which documents the numbers and types of specimens along with their unique identifiers (e.g., two vials of iliac blood, uniquely identified as TX1A and TX1B). The specimens are labeled with the unique DFS laboratory number, unique item/subitem designation and initials of the analyst labeling the evidence (in accordance with QM).

4.2.3 The DUI and toxicology worksheets are used to document additional information such as deficiencies in external packaging, evidence seals, and unusual types or conditions of specimens.

4.3 Storage of Toxicology Biological Evidence

4.3.1 Specimens received by evidence receiving and the toxicology laboratory should, as appropriate, be refrigerated (2-8°C) as soon as possible to preserve their condition. Specimens may also be stored frozen (<-10ºC).

4.3.2 Whenever evidence is not actively being analyzed, it should be stored in secured evidence refrigerators. Access to the refrigerators should be limited to toxicology personnel.

4.3.2.1 Evidence in the process of examination generally will not remain in short term storage for longer than 180 days.

4.3.3 Evidence custody for the receipt into the section and placement into administrative storage shall be documented in accordance with the Quality Manual. Evidence custody for accessioning and sampling of items/subitems shall be documented on toxicology worksheets with FS number, item/sub-item designation, date and analyst's signature.

4.3.3.1 If evidence is transferred between analysts while it is not in the locked toxicology administrative storage, the transfer will be documented on the Toxicology Item Chain of Custody Form. The analysts will indicate the affected items and will document the transfer of custody with their signatures, dates, and times.

4.3.4 Upon completion of a case, OCME and non-implied consent cases are resealed and retained in locked evidence refrigerators until they are returned to the submitting agency. Completed DUI cases are stored at least 120 days to ensure compliance with the Code of Virginia §18.2-268.7 (see DUI evidence handling section).

4.4 DUI Evidence Handling

4.4.1 The following guidelines apply to the receiving, processing, storage and destruction of driving under the influence (DUI) samples pursuant to the Code of Virginia §18.2-266, 268.6 and 268.7 or obtained via search warrant pursuant to the Code of Virginia §18.2-268.5-18.2-268.7.

4.4.2 Evidence Receipt

4.4.2.1 Evidence is typically submitted by mail through a carrier service (United States Postal Service, UPS or Federal Express) and received by DFS Evidence Receiving staff. The unopened container is then transferred to the Toxicology Section. Evidence may also be received directly (hand to hand) by Evidence Receiving staff from submitting officers. All evidence is placed directly into toxicology administrative storage.

4.4.2.2 DUI evidence can be accessioned using one of two methods which shall be documented on the DUI/DUID Summary Worksheet:

Method 1: One DUI kit is accessioned individually and processed to completion as described in Section 4.4.2.3 to Section 4.4.2.7.

Method 2: Several DUI kits are accessioned simultaneously as described in Section 4.4.2.3 to Section 4.4.2.7.

4.4.2.3 The blood vials are removed from the mailing container and the mailing container is discarded. The blood vials are inspected for any discrepancies (e.g., evidence not sealed, vials not provided by DFS or Certificates of Blood Withdrawal (CBWs) not attached to vials). Any discrepancy is noted in the case file.

4.4.2.4 The receiving employee initials both blood vials and affixes the LIMS-generated barcodes with the FS lab number to each vial. If an RFLE is received, then a LIMS-generated barcode with the FS number is affixed to the RFLE and the receiving employee signs and dates the RFLE.

4.4.2.5 The receiving employee shall create a DUI/DUID summary worksheet for each case. The DUI/DUID worksheet includes FS lab number, suspect name, description of number of blood vials and their corresponding vial numbers and the signature of the employee that opened the DUI kit.

4.4.2.6 One of the blood vials is selected for analysis by DFS (typically the blood vial with the greatest quantity of blood). The receiving employee removes the CBW from this vial and labels the CBW with the FS number, their initials and the date. The removed CBWs are stored until the Certificates of Analysis are generated. All blood vials are stored in locked refrigerators.

4.4.2.6.1 If accessioning Method 2 is utilized, an independent person will verify the following:

- The FS Lab # on each blood vial is consistent with the FS Lab # written on the DUI/DUID Summary Worksheet, the RFLE, if available, and the information in FACE.
- The name on the Certificate of Blood Withdrawal is consistent with the name written on the DUI/DUID Summary Worksheet and RFLE.
- The vial number on each blood vial is consistent with the vial numbers written on the DUI/DUID Summary Worksheet.

The verification shall be documented on the DUI/DUID Summary Worksheet with initials and date.

4.4.2.6.2 If additional blood is required for analysis, the second tube may be utilized. If opened, the second CBW will be removed from the vial and labeled with the FS number, initials and the date. The removed CBWs will be stored together.

4.4.2.7 Upon completion of analysis, all blood vials will be sealed and retained in long term storage for a minimum of 120 days after the completion/release of the certificate of analysis (see ¶ 4.3.4). The samples will not be destroyed and will be returned to the submitting agency if the Department receives a written request from the Commonwealth during the examination and subsequent 90 day period.

4.4.3 DUI Evidence Processing and Storage

4.4.3.1 The analyst who analyzes the blood for ethanol content is the analyst that initially breaks the blood vial seal.

4.4.3.2 If the BAC is equal to or greater than 0.10% by weight by volume, a Certificate of Analysis is generated and the stored CBW is affixed to the Certificate of Analysis. The blood vial is then resealed and partnered with the unopened vial of blood in the refrigerator for a minimum of 120 days after the completion/release of the certificate of analysis.

4.4.3.3 If the BAC is less than 0.10% by weight by volume, the case will undergo a series of drugs screens and confirmations.

4.4.3.4 Once drug testing is completed, a Certificate of Analysis is generated and the stored CBW is affixed to the Certificate of Analysis. The blood vial is then resealed and partnered with the second vial of blood in the refrigerator for a minimum of 120 days after the completion/release of the certificate of analysis.

4.4.4 Motions and Court Orders to Transmit Blood Samples to Independent Laboratories

4.4.4.1 All motions and court orders to transmit blood samples are given to a designated DFS employee.

4.4.4.2 Each motion and court order is labeled with the date of receipt, initials of employee receiving motion or court order and corresponding FS lab number (if available).

4.4.4.3 Receipted motions and orders are documented in the DUI excel spreadsheet for electronic tracking.

4.4.4.4 Upon receipt of motion or order, the corresponding blood vials are identified for eventual court ordered release.

4.4.4.5 Motions are stored in the motion file until the corresponding court order is received.

4.4.4.6 Once a court order (signed by a judge) is received, the order is sent to the appropriate DFS personnel who will generate a packet containing a certified mail mailing envelope, partially completed transfer of custody form and a copy of the court order.

4.4.4.7 When the packet is received, a designated toxicology employee or examiner will fill out remaining sections of the transfer of custody form. The sealed blood vials are placed into a DUI shipping container and subsequently placed into the mailing envelope with the copy of the court order and mailed via a carrier service that utilizes a package tracking system which includes documentation confirming delivery. Documentation confirming delivery is attached to the corresponding transfer of custody forms and stored within each case file.

4.4.4.8 When blood samples are mailed, document "transfer out" in LIMS. In comments, document that pursuant to a court order, blood samples were sent to independent laboratory (specify lab).

4.4.5 Requests from Commonwealth's Attorneys to Return Blood Samples.

4.4.5.1 All written requests to return blood samples are given to a designated DFS employee.

4.4.5.2 Each request is labeled with the date of receipt, name or initials of employee receiving the request, and corresponding FS lab number (if available). A copy of the request, and any subsequent withdrawals of that request, if received will be printed and placed in the case file.

4.4.5.3 Received requests are documented in the DUI excel spreadsheet for electronic tracking.

4.4.5.4 Upon receipt of a request, the corresponding blood vials are identified for eventual return. After the 90 day post-examination period has ended and no request for independent testing has been received, the request is sent to the appropriate DFS personnel who will generate a packet containing a partially completed transfer of custody form and a copy of the request and provide it to toxicology staff.

4.4.5.5 When the packet is received, a designated toxicology employee or examiner will fill out remaining sections of the transfer of custody form. The evidence will be prepared for return to the agency via the initial submission process (e.g., hand delivery, mail carrier) unless another method of return has been requested.

4.4.5.5.1 For evidence that is being returned by mail, the sealed blood vials are placed into a DUI shipping container (or other means of containment such as a heat seal bag) and subsequently placed into a mailing envelope with the copy of the request and mailed via a carrier service that utilizes a package tracking system which includes documentation confirming delivery. Documentation confirming delivery is attached to the corresponding transfer of custody forms and stored within each case file. When blood samples are mailed, document "transfer out" in LIMS. In comments, document that pursuant to a request, the blood samples were returned to the submitting agency.

4.4.5.5.2 For evidence that is being returned by hand delivery, the sealed blood vials are placed into a DUI shipping package (or other means of containment such as a heat seal bag) and transferred to the Evidence Receiving section. If the evidence was originally submitted without a Request for Laboratory Examination

(RFLE), a RFLE will be completed by the toxicology employee or examiner. The evidence is then returned to the submitting agency.

4.4.5.5.3 Note: The "DUI shipping container (or other means of containment such as a heat seal bag)" does not need to have a container created for it in LIMS. Items not in a container may be returned to Evidence Receiving or via mail service.

4.4.5.6 In accordance with VA Code 18.2-268.7 B, if requests for both the return of the samples to agencies and to transmit the samples to an independent laboratory are received by toxicology, the request to transmit the samples to an independent laboratory will take priority.

4.4.6 Destroying DUI Evidence

4.4.6.1 If, after 90 days following the release of the CoA, no motions or court orders have been received on a particular case, the blood samples shall be destroyed after 120 days to ensure compliance with the Code of Virginia §18.2-268.7.

4.4.6.2 A deidentified aliquot may be reserved for quality assurance purposes.

4.4.6.3 Place blood vials in biohazard trash.

4.4.6.4 Document destruction of blood vials in LIMS.

5 ESTIMATION OF THE UNCERTAINTY OF MEASUREMENT

5.1 Scope

An estimation of the uncertainty of measurement shall be determined for all analytical procedures in which a quantitative measurement is reported on the Certificate of Analysis.

5.2 Documentation

Calculations related to the reported estimation of measurement uncertainty shall be maintained in the individual case file in which the measurements are made. For compounds with sufficient historical control data, the appropriate uncertainty budget is maintained electronically by the Program Manager. For compounds that do not have sufficient historical control data, "Toxicology Individual Case Sample Calculation of Uncertainty of Measurement" worksheet(s) shall be maintained in the affected individual case file(s).

5.3 Uncertainty of Measurement

5.3.1 Uncertainty Budget

5.3.1.1 Estimations of the uncertainty of measurement shall be conducted and documented using an uncertainty budget.

5.3.1.2 The uncertainty budget for a given procedure shall include both random (Type A) uncertainties and systematic (Type B) uncertainties.

5.3.1.3 Since the uncertainty of measurement is only an estimate, generally uncertainties shall not exceed two significant figures.

5.3.1.4 Calculations used to estimate the uncertainty shall be rounded using conventional rounding rules (see Quality Manual ¶ 5.4.6.3).

5.3.1.5 In order to combine the uncertainty, the uncertainty values should be measured in the same units. Typically, it is beneficial to express all uncertainty values in % to eliminate the necessity to convert measurements to the same units.

5.3.2 Type A Standard Uncertainty

5.3.2.1 Random (Type A) uncertainty results from measurement values being scattered in a random fashion due to laws of chance and thus has a normal or Gaussian shaped distribution.

5.3.2.2 Random (Type A) uncertainty is best determined by historical data of a large number of repeated measurements.

5.3.2.3 Control charts are used to establish the historical standard deviation for common quantitative procedures.

5.3.2.3.1 Examples of historical control data include the following:

- Restek BAC controls
- UTAK controls
- Statewide methanolic controls

5.3.2.3.2 A control chart is a graphical representation of statistically analyzed data and is designed to monitor an analytical process to verify that it is operating within an expected or desired range.

5.3.2.3.3 Once the control chart is established, all future analysis from the same lot of the control sample shall be plotted on the control chart and monitored for trends.

5.3.2.3.4 The relative standard deviation used for uncertainty calculations shall be updated annually.

5.3.2.4 For new methods that lack historical control data, the Section may use repeatability data available from the test method validation and verification data.

5.3.2.5 For quantitative analyses which do not have sufficient historical data, six control replicates shall be analyzed to determine the relative standard deviation of the mean. Control acceptance criteria can be found in ¶ 2.4.5.

5.3.2.5.1 All six control measurements shall be recorded in the "Toxicology Individual Case Sample Calculation of Uncertainty of Measurement" worksheet.

5.3.2.5.1.1 These replicates are considered to be normally distributed with a divisor of 1.

5.3.2.5.2 The control accuracy of ± 20% will also be utilized in calculating the uncertainty of measurement.

5.3.2.5.2.1 Control accuracy is considered to be a rectangular distribution and therefore is divided by the √3.

5.3.2.5.3 The root sum squared method shall be used to calculate the combined uncertainty.

5.3.2.5.4 The expanded uncertainty shall be calculated using the student's t-table for n = 6 (2.65) at 95.45% level of confidence.

5.3.2.6 When multiple measurements are performed on case specimens (e.g., BAC analysis performed in duplicate) the measurements shall be averaged.

5.3.2.6.1 When multiple measurements are performed, as specifically required by the Procedures Manual, uncertainty is calculated using the standard deviation of the mean (σ_{mean}) which is calculated by dividing the historical standard deviation (σ) by the square root of the number of measurements (n).

5.3.2.6.2 In all other instances where multiple measurements are performed a conservative approach shall be taken by using a divisor of 1.

5.3.3 Type B Standard Uncertainty

5.3.3.1 Systematic uncertainty results from the inherent biases in measuring systems and quantitative analytical methods. These uncertainties may be reduced by optimizing the method or measuring system, but can never be completely eliminated.

5.3.3.2 Examples of systematic uncertainties are:

5.3.3.2.1 Using an analytical balance to weigh a powdered standard in the preparation of a calibrator, control or internal standard.

5.3.3.2.2 Preparation of calibrator, internal standard or control solution using 10 mL volumetric flask.

5.3.3.2.3 Using an analytical pipet to prepare calibrator or controls.

5.3.3.2.4 Using a serological pipet to sample 2 mL of specimen.

5.3.3.2.5 Using a Hamilton diluter to prepare calibrators, controls and specimens for BAC analysis by headspace GC.

5.3.3.2.6 Using a repeater pipet to dispense internal standard into all calibrators, controls and case specimens.

5.3.3.2.7 Uncertainty associated with Certificates of Analysis on analytical standards (e.g., Cerilliant Certificate of Analysis on Ethanol-50 standard indicating ethanol concentration of 50 ± 1.55 mg/dL at 95.45% level of confidence). Note: A 95.45% level of confidence is equivalent to 2σ and should be divided by 2 for use in uncertainty calculations (which calculates uncertainty based on 1σ).

5.3.3.3 The scope of the control data used in the estimation of Type A uncertainty (control data gathered by all four DFS toxicology laboratories) already encompasses some associated Type B uncertainties including instruments, maintenance, and analysts.

5.3.3.4 The use of an internal standard for quantitative analysis minimizes other sources of uncertainty including instrumental factors such as the injector, GC column and detector. These miscellaneous uncertainty factors have no significant impact on the overall estimation of uncertainty and are therefore not included in the calculation.

5.3.3.5 Systematic (Type B) uncertainties resulting from measurement bias typically have an equal chance of falling within a range and therefore follow a rectangular or random distribution.

5.3.3.5.1 With rectangular distribution, the range (± a) of the outer limits is used to estimate the standard deviation (σ) using the equation $\sigma = a/\sqrt{3}$.

5.3.3.5.2 For example, a 10 mL volumetric flask has a tolerance of ± 0.02 mL. The calculated uncertainty associated with this measurement is $0.02/\sqrt{3}$ or 0.01.

5.3.4 Combination of uncertainties

Uncertainties are combined using the Root Sum Squares technique

$$U_{combined} = \sqrt{(U_1^2 + U_2^2 + U_3^2 + U_4^2 ....)}$$

5.3.5 Determination of confidence

5.3.5.1 In order to determine the expanded uncertainty, the combined uncertainty is multiplied by the coverage factor (k) using the equation $U_{expanded} = U_{combined} \times k$

5.3.5.2 For routine measurements with a large amount of historical data (n > 30)

5.3.5.2.1 The coverage factor for 95.45% level of confidence is k = 2

5.3.5.2.2 The coverage factor for 99.73% level of confidence is k = 3

5.3.5.3 For analyses with insufficient historical data, a corrected coverage factor (k_{corr}) is used based on the Student's t table.

5.3.5.3.1 For example, for an analysis with no historical control data, a control is analyzed 6 times (degrees of freedom or df = n-1, or 5 in this example).

5.3.5.3.2 Using the Student's t table, k_{corr} value of 2.65 would be used to calculate the expanded uncertainty at 2σ or 95.45% level of confidence.

5.4 Reporting the Estimated Uncertainty of Measurement

The "Toxicology Uncertainty of Measurement Reporting" worksheet shall be used to generate the UOM reporting values.

5.4.1 Since the uncertainty of measurement is only an estimate, it shall be rounded and limited to two significant figures (except for ethanol, acetone, isopropanol, and methanol which are rounded to three (3) decimal places). The reported measurement uncertainty shall be rounded to the same level of significance (i.e., decimal places) as the reported concentration. Measurement uncertainty is reported at a 95.45% level of confidence for all toxicological analyses except ethanol or blood alcohol which is reported at a 99.73% level of confidence.

5.4.1.1 Example 1: Ethanol 0.050 ± 0.003% by weight by volume

5.4.1.2 Example 2: Blood Alcohol Content 0.080 ± 0.004% by weight by volume

5.4.1.3 Example 3: Fentanyl 0.0020 ± 0.0002 mg/L

5.4.2 The following statement shall be included on the Certificate of Analysis UoM attachment – "Measurement uncertainty is reported at a 95.45% level of confidence for all toxicological analyses except blood alcohol or ethanol which is reported at a 99.73% level of confidence".

5.5 Measurement Traceability

Measurement Traceability is an essential element of the Department's measurement assurance program and is required for all measurements where uncertainty of measurement is reported. Measurement traceability for common toxicological analyses is typically accomplished in the following manner.

5.5.1 Measurement Traceability (Drug Compounds):

5.5.1.1 Calibrators: Traceability is established by the procurement of calibrators from an ISO/IEC 17025:2005 and ISO Guide 34:2009 or 17034:2016 accredited reference material provider. The certificate and scope of the provider is kept with the Quality Assurance Coordinator. Certificates of Analysis for each reference material are kept in the laboratory.

5.5.1.2 NIST Traceable Volumetric Flasks: Traceability is maintained for the calibrators by preparing the calibrator stock solutions using NIST traceable volumetric flasks. The certificate of traceability for each piece of glassware is kept in the laboratory.

5.5.1.3 Calibration of Pipettes: Traceability is maintained for the preparation of calibrator stock solutions by utilizing mechanical pipettes externally calibrated annually by an approved ISO/IEC 17025:2005 accredited calibration provider. The certificate and scope of the provider is kept with the Quality Assurance Coordinator. Certificates of calibration for each pipette are kept in the laboratory.

5.5.1.4 Balance Traceability:

5.5.1.4.1 Balance Calibration: The traceability is established by the annual, external calibration of the balances by an ISO/IEC 17025:2005 accredited calibration provider. The certificate and scope of the provider is kept with the Quality Assurance Coordinator. Certificates of calibration for each balance are kept in the laboratory.

5.5.1.4.2 Steel Weight Calibration: Additional traceability is established by the use of steel calibration weights to check the balance calibration monthly. The weights are calibrated every three years by an external, ISO/IEC 17025:2005 accredited calibration provider. The certificate and scope of the provider is kept with the

Quality Assurance Coordinator. Certificates of calibration for each weight and weight set are kept in the laboratory.

5.5.2 Measurement Traceability (Blood Alcohols):

5.5.2.1 Alcohol Calibrators: Traceability is established by the procurement of calibrators from an ISO/IEC 17025:2005 and ISO Guide 34:2009 or 17034:2016 accredited reference material provider. The certificate and scope of the provider is kept with the Quality Assurance Coordinator. Certificates of Analysis for each reference material are kept in the laboratory.

5.5.2.2 Alcohol Controls: Additional traceability may be established by the procurement of controls from an ISO/IEC 17025:2005 and ISO Guide 34:2009 or 17034:2016 accredited reference material provider. The certificate and scope of the provider is kept with the Quality Assurance Coordinator. Certificates of Analysis for each reference material are kept in the laboratory.

5.5.2.3 Calibration of Hamilton Microlab Diluter: Traceability is maintained by utilization of Hamilton Microlab Diluters that are externally calibrated annually by an approved ISO/IEC 17025:2005 accredited calibration provider. The certificate and scope of the provider is kept with the Quality Assurance Coordinator. Certificate of calibration for each diluter are kept in the laboratory.

5.5.3 MeasurementTraceability (Alcoholic Beverages):

5.5.3.1 Alcohol Controls: Traceability is established by the procurement of controls from NIST or an ISO/IEC 17025:2005 and ISO Guide 34:2009 or 17034:2016 accredited reference material provider. The certificate and scope of the provider is kept with the Quality Assurance Coordinator. Certificates of Analysis for each reference material are kept in the laboratory.

5.5.3.2 Calibration of Hamilton Microlab Diluter: Traceability is maintained by utilization of a Hamilton Microlab Diluter that is externally calibrated annually by an approved ISO/IEC 17025:2005 accredited calibration provider. The certificate and scope of the provider is kept with the Quality Assurance Coordinator. Certificates of calibration are maintained in the laboratory.

5.6 References

5.6.1 ASCLD/LAB Policy on Measurement Uncertainty. (AL-PD-3060 Ver. 1.1)

5.6.2 ASCLD/LAB Guidance on the Estimation of Measurement Uncertainty – ANNEX D, Toxicology Testing Discipline. (AL-PD-3065 Ver. 1.0)

6 QUALITY ASSURANCE

6.1 Introduction

6.1.1 The purpose of this section is to provide a uniform Quality Assurance Program for the Section. In combination with the toxicology quality guidelines, it is designed to ensure that the parameters of the testing process are routinely monitored in a manner that maintains the success and reliability of the analytical results.

6.1.2 Since most forensic toxicology specimens are limited in quantity or volume, it is highly desirable to minimize the need for repeat analysis due to the failure of equipment, materials or reagents. The focus of the quality assurance program is to prevent problems before they occur rather than address the failures after they happen.

6.1.3 It is expected that the analyst will report any unacceptable or anomalous behavior of any analytical system immediately to their supervisor. It is further expected that appropriate actions will follow as soon as possible and be properly documented.

6.2 Reagents

6.2.1 Chemicals used in qualitative and quantitative analyses should be of at least ACS reagent grade or better.

6.2.2 Solvents shall be high quality, low residue solvents (e.g., HPLC grade, Omnisolv, Optima etc).

6.2.3 Water used in reagent preparation should be either deionized or reverse osmosis (abbreviated throughout this manual as dH_2O).

6.2.4 Upon receipt of all reagents, chemicals and supplies that could potentially affect test results, the packing slip will be checked for agreement with items received and this review is documented with the signature of the receiver on the packing slip.

6.2.4.1 The signature of the Supervisor / Program Manager / Director on the purchase request signifies that the supply ordered meets the quality specifications listed above and in the toxicology quality guidelines.

6.2.4.2 A copy of the signed purchase request and a signed copy of the packing slip shall be maintained by the Section Supervisor, or designee, for at least six years.

6.2.5 The following information shall be recorded for all purchased reagents and reference materials, either on the bottle or in a log with a reference to the bottle:

6.2.5.1 Date of receipt

6.2.5.2 Date opened

6.2.5.3 Date of verification (if appropriate)

6.2.5.4 The initials of the person opening the bottle

6.2.5.5 The initials of the person performing the verification, if different

6.2.5.6 The expiration date, if necessary

6.2.6 Reagents, chemicals and supplies shall be handled, transported, stored and used in a manner that maintains their quality at an acceptable level. In general, the manufacturer's recommendations for storage conditions as specified on the product label should be followed.

6.2.7 All laboratory prepared reagents, solutions and standards shall be prepared using good laboratory practices.

6.2.8 All prepared reagents shall be documented on the *Reagent Preparation Log* and the forms shall be maintained in a reagent preparation log book (Note: exception for freshly prepared reagents). Reagents that are prepared fresh/consumed on the date of production may be documented on the Toxicology Batch Lot Summary sheet and do not need to be documented on the Reagent Preparation Log. A different final volume of reagent not listed in a particular reagent preparation instruction may be made as long as the final volume prepared is documented on the reagent preparation logbook.

6.2.8.1 A reagent preparation form is created with the individual reagent, preparation instructions and chemical names. Upon completion, the accuracy of the individual form is reviewed by a supervisor or their designee to ensure that the preparation instructions and QC are in compliance with the most current version of the toxicology procedures manual. This review process is documented with initials and date.

6.2.8.2 All reagents must be quality control tested for reliable performance. The QC check is typically performed within a batch of samples as evidenced by the acceptable performance of the calibrators and controls with the particular reagent. As such, the documentation of the QC check is typically the unique FS lab file number that contains the batch analysis raw data.

6.2.8.3 All laboratory prepared reagents/solutions will be clearly labeled to include at a minimum reagent identity, preparer's initials and date of preparation or lot number.

6.2.9 In general, all solutions and reagents (unless otherwise indicated in a specific SOP) may be stored at room temperature for up to 2 years after preparation date or when the solution/reagents fails the quality control check (whichever comes first).

6.2.10 All chemicals and commercial reagents should be replaced when their stated expiration date or shelf life has expired and/or when they fail the quality check.

6.3 Preparation of Blank Blood

6.3.1 Obtain human packed blood cells from a blood bank.

6.3.2 Empty packed blood cells into a large glass jar/container with a lid.

6.3.3 Add approximately 100 mL dH_2O and approximately 100 mL 1% sodium fluoride (10 gm sodium fluoride dissolved in 1 L dH_2O) to achieve similar consistency/fluidity as whole blood.

6.3.4 Label the prepared blank blood with a unique lot number, preparation date and the initials of the preparer.

6.3.5 Analyze each lot of blank blood using an Immunoassay control panel and a base screen. The approving analyst shall clearly note on the data and the bottle any analyte for which the lot is positive.

6.3.6 The lot of blank blood shall not be used for assays that tested positive for the analyte of interest. If additional analyses are to be performed, the blank blood is only acceptable as a negative control if the analyte of interest is shown to be absent during analysis.

6.3.7 Records from blank blood preparation and screening shall be maintained in the section for a minimum of six years.

6.3.8 Store at 2-8°C for up to one year.

6.4 Reference Materials

6.4.1 Reference materials shall be at least of United States Pharmacopeia – National Formulary (USP-NF) quality and are used to prepare calibrators or controls.

6.4.1.1 Whenever possible, certified reference materials should be acquired from vendors accredited to ISO/IEC 17025:2005 and ISO Guide 34:2009 or 17034:2016 and include the supplier's Certificate of Analysis to document traceability, purity, accuracy, precision and homogeneity. If the above requirement cannot be met, it is acceptable to establish traceability through ISO Guide 34:2009 or 17034:2016.

6.4.1.1.1 For instances where traceability cannot be established using certified reference materials other approved vendors may be used to purchase reference materials.

6.4.1.2 Patented reference materials may be obtained directly from the pharmaceutical manufacturer.

6.4.1.3 Reference materials used in casework are considered critical supplies and shall be purchased from manufacturers/vendors/suppliers approved by the Program Manager.

6.4.1.4 The following manufacturers/vendors/suppliers are pre-approved for the purchase of new reference materials:

6.4.1.4.1 Calibrator solutions shall be prepared using certified reference materials from ISO/IEC 17025:2005 and ISO 17034:2016 approved vendors (refer to ¶ 5.5.1.1):

- Cerilliant
- RTC
- Cayman Chemicals
- LGC
- Lipomed
- Restek

6.4.1.4.2 Non-calibrator solutions may be made from the above mentioned list (¶ 6.4.1.4.1) and from the following approved vendors:

- Alltech Associates (Grace – Discovery Sciences)
- UTAK Laboratories, Inc
- Immunalysis
- IL CO controls
- Fisher
- QAS
- SIGMA-ALDRICH
- USP (United States Pharmacopeia)
- Oakwood Chemical

6.4.1.5 Reference materials not verified by the vendor must be verified and documented prior to use or concurrently with casework. Whenever possible, verification should include full spectrum GC-MS analysis with comparison to library spectra and the absence of additional/interfering chromatographic peaks or the use of other analytical techniques as necessary (HPLC-DAD, LCMSMS or UV-VIS spectrophotometry) to generate suitable definitive instrumental data.

6.4.1.5.1 Verification data should be labeled with analyst's initials and date.

6.4.1.5.2 Store the verification data in the drug standard logbook containing corresponding Certificates of Analysis from drug manufacturers. Drug manufacturer Certificates of Analysis can be stored electronically in appropriately labeled folders.

6.4.1.5.3 Due to the nature of the work within the Section, verification may be performed within a batch of samples when the standard is used as a calibrator or control. As such, the documentation of verification may be stored with the unique FS lab file

number that contains the batch analysis raw data with the FS number noted within the drug standard records.

6.4.1.6 Reference materials shall be stored in a manner that maintains their quality. In general, powders are stored at room temperature, aqueous solutions are stored at 2-8°C and methanolic/acetonitrile standards are stored at -10 to -20°C, unless otherwise indicated by the supplier or in a specific method SOP. Allow all reference materials and reagents to come to room temperature prior to starting procedures.

6.4.1.7 DFS assigned expiration dates for multi-component reference material solutions shall be set for two years from date of preparation or for the earliest expiration of a reference material component, whichever is shorter. Solutions may be retested to extend the DFS expiration date for at most one additional year.

6.4.2 Records

6.4.2.1 The *Drug Inventory Log* will be used to track purchased drug reference materials. This form may be stored electronically or in hardcopy (laboratories should choose only one option and not use the storage formats interchangeably).

6.4.2.2 The *Drug Stock Standard Preparation Log* will be used to track the preparation of stock standards. If the stock standard is used as a calibrator, or used to create a calibrator, the serial number of the calibrated glassware used shall be documented on the preparation log.

6.4.2.3 The *Multi-component Standard, Calibrator and Control Preparation Log* will be used to track the preparation and verification of multi-component standard, calibrator and control solutions. If the solution is used as a calibrator, or used to create a calibrator, the serial number of the calibrated glassware used shall be documented on the preparation log.

6.5 Reference Collections

6.5.1 Reference collections of data or materials used for identification, comparison or interpretation shall be fully documented, uniquely identified and properly controlled.

6.5.2 Purchased data libraries (reference collections) are fully documented and uniquely identified. No changes may be made to purchased reference collections. Examples of such libraries include GC-MS NIST, GC-MS Pfleger/Maurer/Weber, and Wiley.

6.5.3 Data libraries obtained from reputable forensic sources are fully documented and uniquely identified. No changes may be made to these reference collections. The addition or removal of forensic libraries must be approved by the Program Manager. Current forensic libraries approved for use:

- GC-MS AAFS
- GC-MS NIST
- GC-MS Wiley
- GC-MS ENFSI
- GC-MS TIAFT
- GC-MS SWGDRUG
- GC-MS Cayman Chemical
- GC-MS Pfleger/Maurer/Weber

6.5.4 For in-house libraries, each entry is automatically identified by a unique tracking number generated by the instrument software. These libraries should be generated or modified by an instrument operator or by a designee of the Section Supervisor.

6.5.4.1 At a minimum the following information should be included with each new entry into in-house data libraries:

- Compound name
- Drug standard identifier (lot number)
- Date
- Initials of person entering data

6.5.5 A reference collection of drug standards is maintained for use as reference materials. These are uniquely identified through the use of laboratory lot/tracking numbers in addition to the manufacturer's lot number. Access to drug standards and records is limited to section members.

6.6 Balances

6.6.1 All analytical balances will be checked monthly for accuracy using Class 1 weights or better. Record the weights in the balance log book with the date and analyst's initials.

6.6.2 Weights used to check balance accuracy shall be re-certified every three years by an ISO/IEC 17025:2005 accredited vendor whose scope of accreditation covers the certification performed.

6.6.3 The below listed balances are intended as examples of a balance class of type with appropriate check weights. If the individual balance does not fit into these categories, use three weights within its range or as approved by the Program Manager.

Balance Type	Balance Examples	Check Weights
Analytical (dual range)	Mettler XS 105	0.01000 (± 0.00005) g 20.00000 (± 0.00020) g 50.0000 (± 0.0005) g 100.0000 (± 0.0005) g
Analytical	Mettler AE 160 Mettler AG204 Ohaus E-10640	50.0 (± 0.2) mg 100.0 (± 0.2) mg 1000.0 (± 0.2) mg
Top Loading (± 0.01) gram	Mettler PE 2000 Mettler PB303 Mettler PE1600 Sartorius 2100	5.00 (± 0.02) g 10.00 (± 0.03) g 100.00 (± 0.03) g
Top Loading (± 0.001) gram	Mettler PC303 Ohaus Explorer	0.500 (± 0.002) g 1.000 (± 0.002) g 100.000 (± 0.005) g

6.6.4 Accuracy must be established for monthly balance checks or after a balance has been put into service after purchase, maintenance or repair. The following are guidelines for performing balance checks.

6.6.4.1 The check weights listed in the table in 6.6.3 are weighed and recorded on the balance logsheet.

6.6.4.2 The accuracy of each weight should meet the criteria in 6.6.3

6.6.4.3 If the accuracy of a weight is outside the acceptable range, ensure that the balance is level and clean prior to rechecking.

6.6.4.4 Perform balance performance check again. If the balance accuracy fails again, use the internal calibration function to recalibrate balance. Repeat balance check. Record all pre and post calibration measurements on logsheet.

6.6.4.5 If, after the above mentioned actions, the result of the balance check is still outside of the acceptable range, re-calibrate the balance for a second time. Repeat balance check. If it fails again, the balance shall be taken out of service (and labeled as such) until maintenance and/or calibration are performed by a qualified and approved vendor.

6.6.4.5.1 Record the service call on the analytical balance QC sheet kept with each analytical balance.

6.6.4.5.2 If the balance is taken out of service for repair/maintenance, perform a balance check prior to putting the balance back into service.

6.6.5 All balances are cleaned, serviced and calibrated annually by an outside vendor that is accredited to ISO/IEC 17025:2005 and whose scope of accreditation covers the calibration performed. Record the service call on the analytical balance QC sheet kept with each analytical balance. Each balance shall be labeled with the date of calibration and when the next calibration is due.

6.6.6 Minimum balance loads:

6.6.6.1 5-Place balance = 0.00450 gram

6.6.6.2 4-Place balance = 0.0300 gram

6.6.6.3 3-Place Balance = 0.150 gram

6.6.6.4 2-Place balance = 0.90 gram

6.6.6.5 High Capacity (g) Balance = 27.0 grams

6.7 pH Meters

6.7.1 Calibrate the pH meter prior to each use. Refer to the individual pH meter's instrument manual for these procedures.

6.7.1.1 The reference buffers chosen should bracket the expected pH value range of the solution, if possible.

6.7.1.2 The pH values must be within ± 0.1 units of the pH value stated on each individual reference buffer's labeling.

6.7.1.3 If the calibration values are within the accepted limits, the pH meter is ready to use for reagents.

6.7.1.4 If the calibration values are not within the accepted limits, rerun and/or troubleshoot as necessary.

6.7.2 Rinse the electrode with dH_2O, as appropriate.

6.7.3 Reference buffers shall be replaced when they expire.

6.7.3.1 Keep the buffer bottle tightly sealed and free of contamination.

6.7.3.2 Do not reuse an aliquot of buffer or return it to the original bottle.

6.7.4 Refer to the pH meter instrument manuals for recommendations on proper use and storage, good laboratory practices, correct applications, problem samples and trouble shooting.

6.7.5 For non-critical pH measurements, an approximate pH may be determined using pH paper.

6.8 Pipettes

6.8.1 Fixed volume, variable volume, multichannel repeater pipettes and Hamilton diluters shall have their calibration evaluated and certified annually by an approved ISO/IEC 17025:2005 accredited vendor whose scope of accreditation covers the calibration performed.

6.8.2 Maintain repair documentation and calibration certificates generated by the vendor in the pipette logbook. The calibration certificates demonstrate that the pipette has passed QC prior to being placed into or back into service.

6.8.3 As needed, clean the inside and outside of pipettes with isopropanol and check the seal.

6.8.4 If a pipette appears to be out of calibration between normally scheduled performance/calibration checks, the pipette will be sent to an authorized vendor for repair.

6.8.4.1 The maintenance/repair shall be documented in the pipette log book along with the calibration certificate generated by the vendor.

6.8.5 All pipettes shall be uniquely identified and appropriately labeled with the date of calibration and when the next calibration is due.

6.9 Refrigerators/Freezers

All refrigerators and freezers that are used to store biological evidence or critical reagents should be monitored triweekly to ensure the appropriate storage temperature. This includes refrigerators and freezers within the section and those used by evidence receiving for the temporary storage of toxicology evidence.

6.9.1 Post a temperature log on each refrigerator or freezer.

6.9.2 For refrigerators/freezers without a digital external temperature monitor, place a thermometer in each refrigerator or freezer. All thermometers should be NIST traceable or verified annually against a NIST traceable thermometer (see thermometer section).

6.9.3 Read and record the thermometer temperature of refrigerators and freezers triweekly (Monday, Wednesday and Friday). If the temperature checks cannot be performed on Monday, Wednesday or Friday due to a holiday, they should be made on the first regularly scheduled workday following the holiday.

6.9.4 Record temperatures on the temperature log along with the initials of the staff member performing the temperature check.

6.9.4.1 For refrigerators, the temperature should fall between 2-8°C.

6.9.4.2 For freezers, the temperature should fall below -10°C.

6.9.5 If the temperature should fall just outside the acceptable range, the thermostat should be adjusted accordingly to bring the temperature back into the acceptable range. Document this adjustment on the temperature log and continue to monitor the temperature of the unit daily for the following week to ensure the thermostat adjustment was effective.

6.9.6 For extreme temperature changes (e.g., freezer above 0°C, refrigerator below 0° C or greater than room temperature), all biological evidence and critical reagents should be removed immediately from the affected unit and placed in alternative refrigerators and/or freezers. The refrigerator or freezer should be placed out of service, labeled as such until it can be repaired and the repair should be documented on the temperature log.

6.9.6.1 The quality of critical reagents exposed to extreme temperatures may be compromised and the affected reagents should undergo a performance check or verification prior to their use on casework.

6.9.7 Maintain temperature logs for all refrigerators and freezers for at least six years.

6.10 Heat Blocks

6.10.1 Heat blocks are generally used for the evaporation or derivatization of samples.

6.10.2 With each use, the temperature of the heat block should be checked with a thermometer to ensure the temperature falls within the recommended approximate temperature range.

6.10.2.1 The thermometer should be NIST traceable or verified annually against a NIST traceable thermometer.

6.10.2.2 Adjust the thermostat as necessary to achieve the desired temperature.

6.10.2.3 The temperature should fall within 2°C of the recommended temperature range.

6.10.2.4 If the correct temperature cannot be achieved, remove the heat block from service and label it as such until it can be repaired. Document the repair in the heat block instrument logbook.

6.10.3 Documentation of the performance of the heat block is evidenced by the acceptable performance of the calibrators and controls with each batch analysis.

6.11 Evaporators

6.11.1 The temperature of the evaporator should be checked using a thermometer to ensure the temperature falls within the recommended approximate temperature range.

6.11.1.1 The thermometer should be NIST traceable or verified annually against a NIST traceable thermometer.

6.11.1.2 Adjust the thermostat as necessary to achieve the desired temperature.

6.11.1.3 The temperature should fall within 2°C of the recommended temperature range.

6.11.1.4 If the correct temperature cannot be achieved, remove the evaporator from service and label it as such until it can be repaired. Document the repair in the evaporator instrument logbook.

6.11.2 Documentation of the performance of the evaporator is evidenced by the acceptable performance of the calibrators and controls with each batch analysis.

6.11.3 Check the nitrogen gas supply and replace as needed.

6.11.4 Add water and anti-algae drops as needed (to turbovap).

6.12 Centrifuges

6.12.1 All recommended centrifuge speeds are approximate in order to achieve the appropriate separation of layers. Therefore, no intermediate tachometer checks to verify the speed of the rotor are required.

6.12.2 Clean centrifuge as needed.

6.12.3 If a centrifuge is taken off line for repair/maintenance, it should be labeled as out of service until it is repaired. Document repair/maintenance in the centrifuge maintenance log.

6.13 Thermometers

6.13.1 All thermometers used to check temperatures on refrigerators, freezers, heat blocks and evaporators should be certified by NIST or NIST traceable; or they may be checked for accuracy against a NIST or NIST taceable thermometer annually.

6.13.2 NIST or NIST traceable thermometers are good for 2 years from their original date of calibration. Purchase a new NIST or NIST traceable thermometer when the unit needs to be recalibrated. Maintain documentation of the calibration of the NIST traceable thermometer.

6.13.3 The following are guidelines to verify thermometer accuracy against a NIST or NIST traceable thermometer.

6.13.3.1 Tag each thermometer with an identifying number.

6.13.3.2 Thermometers should be checked for accuracy at temperatures similar to those of their intended use.

6.13.3.3 The easiest way to verify the accuracy of a thermometer is to simply compare its reading to that of a NIST or NIST traceable thermometer under the same conditions (e.g., place a NIST or NIST traceable thermometer in refrigerator with the thermometer requiring verification). Record the temperatures of the thermometer and the NIST or NIST traceable thermometer.

6.13.3.4 To verify the accuracy of digital thermometers on refrigerators and freezers, simply place a NIST or NIST traceable thermometer in the refrigerator or freezer. Record the temperatures of the thermometer and the digital readout.

6.13.3.5 To verify the accuracy of multiple thermometers at one time use one of the following procedures:

6.13.3.5.1 For thermometers used in the range of 2-8°C (refrigerator), prepare an ice/water slurry in a beaker. Place a NIST traceable thermometer and all other thermometers in the beaker. Record the temperature of each thermometer.

6.13.3.5.2 For thermometers used below 0°C, prepare an ice/water slurry in a beaker and add salt to lower the temperature below 0°C. Place a NIST traceable thermometer and all other thermometers in the beaker. Record the temperature of each thermometer.

6.13.3.5.3 For thermometers used in the high temperature range (heat blocks and evaporators), partially fill the wells of a heat block with sand or fill a test tube with glycerol. Turn on the heat block to be in range of 50-80°C. Place a NIST traceable thermometer and all other thermometers in the heat block or glycerol. Record the temperature of each thermometer.

6.13.4 All thermometers should be within ± 2°C of a NIST or NIST traceable thermometer within the range of intended use. Any thermometers that vary more than 2°C from a NIST or NIST traceable thermometer will not be used.

6.13.5 Record the date/initials of the annual thermometer accuracy check on the refrigerator log and on the unique identifying tag on each thermometer.

6.14 Incubators (Ovens)

Incubators (ovens) are only used to dry glassware therefore it is not necessary to verify the temperature of the oven.

6.15 Fume Hoods and Biological Safety Cabinets

Refer to the Department Safety Manual.

6.16 UV-VIS Spectrophotometer

6.16.1 Day-of-Use

6.16.1.1 Turn on the instrument and visually verify that the automatic initialization operation passes (displayed on instrument screen). Record initialization results in the instrument log with the date and initials.

6.16.1.2 Allow the instrument to warm up for approximately one hour.

6.16.1.3 Perform a Baseline Correction from 675 nm – 475 nm. If the baseline is not stable at 0 Absorbance units, perform the Instrument Baseline Correction.

6.16.1.4 Initialize the 6-cell positioner. This function can be found on the "Attachments" tab of the Edit Method window of the UVProbe software.

6.16.1.5 As necessary, clean sample compartment.

6.16.2 Semi-annually

6.16.2.1 Check wavelength accuracy at two characteristic peaks of deuterium light at 486.0 nm and 656.1 nm (refer to the instrument maintenance instruction in the UVProbe Software for details).

6.16.2.1.1 Acceptance criteria

6.16.2.1.1.1 The 486.0 nm peak should fall within 485.5 nm and 486.5 nm.

6.16.2.1.1.2 The 656.1 nm peak should fall within 655.6 nm and 656.6 nm.

6.16.2.1.1.3 Maintain spectrum peak pick reports of wavelength accuracy checks in the instrument log.

6.16.2.1.1.4 If the wavelengths do not fall within the specified ranges, contact the instrument manufacturer service representative.

6.16.2.2 Check wavelength accuracy using a NIST traceable holmium oxide wavelength standard.

6.16.2.2.1 Collect and print a spectrum of the holmium oxide standard. Compare peak wavelengths to those listed on the calibration certificate of the standard. Record the differences on the printout.

6.16.2.2.2 Record acceptable results in the instrument log and maintain the printout.

6.16.2.2.3 The holmium oxide standard shall be recertified prior to the expiration date listed on the calibration certificate.

6.16.2.2.4 Acceptance criteria

6.16.2.2.4.1 Bands shall fall within 2 nm of certified target wavelengths.

6.16.2.2.4.2 If the wavelengths do not fall within 2 nm of target wavelength, remove the instrument from service and contact the instrument manufacturer service representative.

6.16.3 Every two years

6.16.3.1 Have the instrument manufacturer perform a certified performance test (PT) on the instrument. This PT includes the following:

6.16.3.1.1 Wavelength setting test

6.16.3.1.2 Wavelength Accuracy Test (using NIST certified Hg lamp)

6.16.3.1.3 Photometric Accuracy Test (using NIST traceable photometric accuracy filters)

6.16.3.2 Maintain PT documentation in the instrument log.

6.17 Gas Chromatographs

6.17.1 Most toxicology procedures are performed in batch and therefore most maintenance procedures are performed prior to running a batch of samples. Record all maintenance in the Instrument Log with the date and initials.

6.17.2 Day-of-Use

6.17.2.1 For MSD, perform Autotune. A hardcopy of the Autotune shall be maintained for at least six years. Autotune results shall meet the following criteria:

- The mass assignments shown in the upper profile part of the display should be within ± 0.2 amu of 69, 219 and 502.
- Inspect the mass peaks in the upper profile part of the display for good peak shape (no peak splitting and resolution between mass 502 and 503).
- The peak widths (PW) of the three peaks should be 0.6 ± 0.1 amu.
- The isotope ratio figures (indicating the relative abundances of the naturally occurring isotopes) should be within 25% of the theoretical values of 1.08 for m/z 69, 4.32 for m/z 219 and 10.09 for m/z 502.
- Air and water leaks (masses 28 and 18) should be minimal.

6.17.2.2 Run a Performance Check (e.g., a specific instrument check mix or a batch calibrator). Maintain a hardcopy or digital version in the laboratory.

6.17.3 As Needed

6.17.3.1 The following should be replaced as needed:

- Septa/Merlin Microseal
- Liner
- Gold seal
- Gap column
- Syringe
- Columns
- Gas filters

6.17.3.2 Check and replace gas cylinders.

6.17.3.3 Clip front portion of column and reinstall.

6.17.3.4 Silanize injection port liner (excluding instruments with NPD). An example of instrument parameter which may be used:

- Injector: Split Mode
- Gas Saver: Off
- Split Ratio: 200:1
- Injection port: 200ºC
- Oven: 200ºC
- Silanizing reagent: Silyl-8
- Injection volume: 2 µL
- Condition column after injection

6.17.3.5 Condition column

6.17.3.5.1 Short method example: Hold oven temperature 10 degrees above Final Temperature for 15 minutes

6.17.3.5.2 Long method example: Hold oven temperature 300 degrees for 600 minutes (overnight)

6.17.3.6 Detector maintenance

6.17.3.6.1 Clean MSD source

6.17.3.6.2 Replace NPD bead

6.17.3.6.3 Replace NPD ceramics

6.17.3.6.4 Clean FID

6.17.3.6.5 Replace or clean jet

6.17.4 Every six months

6.17.4.1 Computer maintenance

6.17.4.1.1 Archive methods, macros and data files onto long term storage media.

6.17.4.1.2 Once archived, data files and sequence files more than one month old may be deleted from the hard drive.

6.17.4.1.3 Perform appropriate disk clean-up and defragment the hard drive.

6.17.5 Annually

6.17.5.1 Schedule an on-site preventive maintenance service call as per the service agreement. Preventive maintenance shall be performed on instruments not covered under service contract in accordance with the manufacturer's recommendations.

6.17.6 After the instrument has been shut down or significant maintenance has been performed, verify that the instrument is fit for use by running a check mix solution, positive control or calibrator to ensure appropriate sensitivity, chromatography and separation of the components of the mixture.

6.17.6.1 Retain instrument verification documentation in the instrument logbook.

6.17.7 Hydrogen generator

6.17.7.1 Add dH_20 weekly or as needed.

6.17.7.2 Change moisture filter as needed.

6.17.7.3 Change deionizer bags at least annually.

6.17.8 Zero air generator

6.17.8.1 Replace filter cartridges annually.

6.17.8.2 Replace catalyst module, as needed.

6.18 HPLC Systems

6.18.1 Record all maintenance in the Instrument Log with the date and initials.

6.18.2 Day of Use – Before Use

6.18.2.1 Prepare fresh aqueous buffer solutions, as needed and add to a clean reservoir.

6.18.2.2 Run a Performance Check (e.g., a specific instrument check mix or a batch calibrator). Maintain a hardcopy in the laboratory.

6.18.3 Day of Use – After Use

6.18.3.1 Wash column by substituting water for aqueous buffer and running a gradient.

6.18.3.2 Store column by running gradient until a high percentage of organic solvent is achieved (e.g., 95% Acetonitrile:Water).

6.18.4 As Needed

6.18.4.1 The following should be replaced as needed:

- PTFE filter
- Active inlet valve
- ALS needle and seat
- In-line filter
- Column

6.18.4.2 Replace and reseat pump seals

6.18.4.3 Backflush column

6.18.5 Every Six Months

Perform Computer Maintenance outlined above in ¶ 6.17.5.1.

6.18.6 Annually

Schedule an on-site preventive maintenance service call as per the service agreement.

6.18.7 Water purifier

6.18.7.1 Perform maintenance as indicated by the manufacturer.

6.19 Tandem Mass Spectrometer (QQQ)

6.19.1 Most toxicology procedures are performed in batch and therefore most maintenance procedures are performed prior to running a batch of samples. Record all maintenance in the Instrument Log with the date and initials.

6.19.2 Day-of-Use

6.19.2.1 For QQQ, perform Checktune. A file containing the Checktune parameters will be maintained in an electronic format for a minimum of six years. The Checktune report shall be assessed for the following:

6.19.2.1.1 The mass-to-charge (m/z) assignments and the full-width-at-half-maximum (FWHM) should be within the stated tolerance of the Checktune parameters.

6.19.2.1.1.1 Exceptions are made for the two highest m/z assignments.

6.19.2.1.1.2 If any of the other m/z assignments or FWHM are out of tolerance, the operator shall adjust the "Gain and Offset" in the manual tune mode. After completing this adjustment, the operator shall repeat the Checktune.

6.19.2.1.1.3 If the Checktune repeatedly fails, the operator shall run an Autotune.

6.19.2.1.2 The abundances should be compared to previous Checktunes and reviewed for any substantial deviations.

6.19.2.2 Clean the source, including the spray shield and spray chamber. The capillary cap can be cleaned, as needed.

6.19.2.3 Check solvent levels and replace as needed.

6.19.2.4 Run a Performance Check (e.g., a specific instrument check mix or a batch calibrator). Maintain a copy of the data in an electronic format for a minimum of six years.

6.19.3 As Needed

Check and replace gas cylinders and gas filters

6.19.4 Weekly

6.19.4.1 Check the demister on the rough pump and drain as needed.

6.19.4.2 Check the water drain reservoir from nitrogen generator and empty as needed.

6.19.5 Monthly

6.19.5.1 Perform an Autotune.

The operator shall ensure that m/z assignments, fragmentation patterns, and isotope separation are satisfactory. An electronic copy of the Autotune shall be maintained for a minimum of six years.

6.19.6 Every six months

6.19.6.1 Computer maintenance

6.19.6.1.1 Archive methods and data files onto long term storage media.

6.19.6.1.2 Once archived, data files and sequence files more than one month old may be deleted from the hard drive.

6.19.7 Annually

Schedule an on-site preventive maintenance service call as per the service agreement.

6.19.8 After the instrument has been shut down or significant maintenance has been performed, verify that the instrument is fit for use by running a check mix solution, positive control or calibrator to ensure appropriate sensitivity, chromatography and separation of the components of the mixture.

6.19.8.1 Notate instrument verification in the instrument logbook and retain documentation in an electronic format.

6.19.9 Nitrogen generator

Change moisture filter as needed.

6.20 New Instrument Installation

6.20.1 Obtain documentation from the instrument service representative that demonstrates that the instrument performs to the manufacturer's specification.

6.20.2 Load methods, macros and libraries and test their functionality.

6.20.3 Perform self check or autotune (GC-MS, QQQ), as needed.

6.20.4 After methods have been loaded or created, run check solutions, positive controls or calibrators to demonstrate the instrument is fit for use (e.g., appropriate sensitivity, specificity, accuracy, precision, chromatography or identification of the components of the mixture).

6.20.5 Archive methods and data analysis macros to suitable long-term storage media.

6.20.6 Retain instrument verification documentation in the instrument logbook.

6.20.7 A summary of the verification shall be sent to the Program Manager for approval prior to placing the new instrument into service.

6.20.8 If the instrument does not meet expectations or acceptance criteria, label it as "not in service" and notify the Program Manager as soon as possible.

6.21 Immunalysis ELISA System

6.21.1 TECAN System (Note: annual PMs may be performed by the manufacturer while the equipment is under warranty)

6.21.1.1 Daily (with each use)

6.21.1.1.1 Clean the surfaces with isopropanol.

6.21.1.1.2 Clean the probe with isopropanol.

6.21.1.1.3 Check fluid levels and empty waste containers.

6.21.1.2 Weekly

6.21.1.2.1 Check tubing for leaks.

6.21.1.2.2 Clean liquid reservoirs with mild soap solution and rinse thoroughly.

6.21.1.3 Monthly

6.21.1.3.1 Perform acid/base wash of system.

6.21.1.3.1.1 Prepare 100 mL of a 1 N HCl solution and place the system intake tubing into the acid solution.

6.21.1.3.1.2 Flush the instrument with approximately 50 mL of acidic solution.

6.21.1.3.1.3 Let the solution stand in system for approximately 15-20 minutes.

6.21.1.3.1.4 Place the system intake tubing into a beaker containing dH_20 and flush the instrument with approximately 50 mL of dH_2O.

6.21.1.3.1.5 Prepare a 1 N NaOH solution and place the system intake tubing into the basic solution.

6.21.1.3.1.6 Flush the instrument with approximately 50 mL of basic solution.

6.21.1.3.1.7 Let the solution stand in the system for approximately 15-20 minutes.

6.21.1.3.1.8 Remove tubing from basic solution and place into beaker containing dH_20.

6.21.1.3.1.9 Flush the instrument with approximately 50 mL dH_20.

6.21.1.3.1.10 Replace the intake tubing back into the liquid reservoir and flush instrument 3 times with approximately 50 mL dH_2O.

6.21.1.4 Annually

6.21.1.4.1 Replace sampling probe, syringe cap, pump valves, probe tubing, reagent tubing and waste tubing as needed.

6.21.1.4.2 Perform performance check, only if the annual preventive maintenance check does not include a precision test of the dispensing system.

6.21.1.4.2.1 Place Fisher pH 7 buffer (yellow solution) in reagent, TMB or substrate trough.

6.21.1.4.2.2 Pipet 100 µL of the solution into all wells of a blank 96 well plate.

6.21.1.4.2.3 Read absorbance at 450 nm. Calculate CV for each absorbance reading.

6.21.1.4.2.4 Turn plate 180° and reread plate. Calculate CV for each absorbance reading.

6.21.1.4.2.5 The CV for each well across the plate should be less than 10%. If the CV exceeds 10%, contact Immunalysis for service.

6.21.2 Plate washer

6.21.2.1 Daily (with each use)

6.21.2.1.1 Clean surfaces with isopropanol.

6.21.2.1.2 Perform Rinse/Night with dH_2O daily.

6.21.2.2 Monthly

6.21.2.2.1 Disassemble manifold and sonicate in methanol. Reassemble. (as needed)

6.21.2.2.2 Clean dispensing needles with cleaning needles.

6.21.2.2.3 Check liquid filters in wash bottles for particles and rinse liquid filters with dH_2O.

6.21.2.2.4 As needed, acid/base wash the plate washer mirroring the procedure listed for the Tecan (¶ 6.21.1.3.1).

6.21.2.3 Annually

6.21.2.3.1 Disinfect instrument with 10% bleach solution.

6.21.2.3.2 Replace all aspirating and dispensing tubes, as needed.

6.21.2.3.3 Replace all liquid filters, as needed.

6.21.2.3.4 Replace manifold sealing, as needed.

6.21.2.3.5 Recalibrate the dispensing pump, as needed.

6.21.3 Plate reader

6.21.3.1 Daily (with each use)

Clean surfaces with isopropanol.

6.21.3.2 Every six months

Clean the filters.

6.21.3.3 Yearly – only required when an instrument is not covered under the manufacturer's warranty (typically three years after purchase)

Perform the QC Pac 2 Test. This test provides an automated check of the reader performance including accuracy, linearity, precision and alignment with NIST traceable standards. It also detects damaged or mislabeled filters.

6.21.3.4 Every four years (manufacturer service technician)

6.21.3.4.1 Replace the lamp and filters.

6.21.3.4.2 Perform extended operational quality check.

6.22 Glassware

Volumetric glassware used to prepare calibrators shall be NIST traceable and visually inspected prior to each use.

7 ALCOHOLS BY HEADSPACE GAS CHROMATOGRAPHY

7.1 Summary

An aliquot of each biological specimen is diluted semi-automatically with an internal standard solution into a glass vial, sealed, and placed in a heated headspace autosampler. Positive cases are confirmed in a second run. The concentration of ethanol or other volatiles in a dilute aqueous biological sample is directly proportional to the concentration of these compounds in the gas phase (headspace). A portion of the resultant headspace vapor above the liquid is automatically injected into a dual column gas chromatograph (GC) equipped with dual flame ionization detectors (FID). Ethanol, methanol, acetone and isopropanol are identified by retention time and the concentrations of these volatile compounds are calculated automatically by the software based on linear regression of the calibration curve.

7.2 Specimen Requirements

Approximately 100 µL of blood or other fluids or 1-2 g tissue.

7.3 Reagents and Standards

- NIST traceable ethanol standards for use as calibrators (0.20% and 0.50% by weight by volume (w/v)), stored at 2-8°C.
- NIST traceable multicomponent alcohol mixes for use as calibrators containing 0.01%, 0.05% and 0.10% w/v acetone, methanol, ethanol and isopropanol, stored at 2-8°C.
- Absolute 200 proof ethanol, stored at 2-8°C.
- Methanol
- Isopropanol
- Acetone
- n-Propanol
- NIST traceable ethanol standards for use as controls (0.05%, 0.08%, 0.10%, 0.20%, and 0.30% w/v), stored at 2-8°C.

7.4 Calibrators, Controls and Internal Standards

7.4.1 Calibrators

7.4.1.1 Calibrators may be purchased as NIST traceable standards or prepared in-house. If in-house controls are utilized, calibrators must be NIST traceable.

7.4.1.2 Purchased calibrators

7.4.1.2.1 0.50% and 0.20% ethanol standards.

7.4.1.2.2 0.10%, 0.05% and 0.01% multicomponent alcohol mix standards.

7.4.1.3 Preparation of in-house calibrators

7.4.1.3.1 0.50% Ethanol calibrator. Pipet 634 µL of absolute ethanol into a 100 mL volumetric flask containing dH2O and qs to volume with dH2O. Store at 2-8° C for up to one year.

7.4.1.3.2 0.20% Ethanol. Pipet 253 µL of absolute ethanol, into a 100 mL volumetric flask containing dH2O and qs to volume with dH2O. Store at 2-8° C for up to one year.

7.4.1.3.3 0.10% Ethanol and mixed volatile calibrator. Pipet 126 µL of absolute ethanol, acetone, isopropanol and methanol into a 100 mL volumetric flask containing dH2O and qs to volume with dH2O. Store at 2-8° C for up to one year.

7.4.1.3.4 0.05% Ethanol and mixed volatile calibrator. Pipet 63 µL of absolute ethanol, acetone, isopropanol and methanol into a 100 mL volumetric flask containing dH2O and qs to volume with dH2O. Store at 2-8° C for up to one year.

7.4.1.3.5 0.01% Ethanol and mixed volatile calibrator. Pipet 12.7 µL of absolute ethanol, acetone, isopropanol and methanol into a 100 mL volumetric flask containing dH2O and qs to volume with dH2O. Store at 2-8° C for up to one year.

7.4.2 Internal standard (IS) preparation: n-propanol can be a putrefactive product. If n-propanol contamination is suspected, other internal standards such as methyl ethyl ketone or t-butanol may be used. Document this exception in the case file with a MFR.

7.4.2.1 0.03% (v/v) n-propanol internal standard solution. Pipet 300 µL n-propanol into a 1 L volumetric flask and qs to volume with dH_2O. Store at room temperature for up to one year.

7.4.3 Controls

7.4.3.1 Positive controls within the batch must be purchased or prepared from a source separate from the calibrators. If in-house calibrators are utilized, controls must be NIST traceable.

7.4.3.2 NIST traceable ethanol controls: 0.10%, 0.20%, 0.30%, 0.05% and 0.08% w/v.

7.4.3.3 NIST traceable multicomponent alcohol control containing 0.05% w/v acetone, methanol, ethanol and isopropanol. In-house prepared multicomponent controls may be substituted.

7.4.3.4 0.05% Multicomponent alcohol control. Pipet 63 µL of acetone, isopropanol and methanol into a 100 mL volumetric flask containing dH_2O and qs to volume with dH_2O. Store at 2-8° C for up to one year.

7.4.3.5 Negative control: prepared from dH_2O or blood.

7.5 Apparatus

7.5.1 Gas chromatograph with data system, dual flame ionization detectors and a headspace autosampler

7.5.2 Columns. Restek Rtx-BAC 1 and Rtx-BAC 2 or Agilent DB-ALC1 and Agilent DB-ALC2 capillary columns or Agilent DB-BAC1 UI and DB-BAC2 UI capillary columns

7.5.3 Glass 20 mL (23 x 75 mm) headspace vials with Teflon or Butyl septa and aluminum seals

7.5.4 Hamilton Microlab Diluter

7.5.5 Vial seal crimper

7.5.6 Test tubes or sample cups

7.6 Procedure

At a minimum, calibrators, controls, and case specimens are analyzed singly. If a case specimen is positive, it will be realiquotted and confirmed on a second run. For post-mortem cases, confirmatory analyses run on an additional, different biological specimen or tissue may be run in duplicate on the same analytical run (see ¶ 2.2.4.3). Negative specimens may be reported with the quantitative results from the initial run.

7.6.1 Allow all case samples, calibrators and controls to come to room temperature before preparing the aliquots.

7.6.2 Mix biological fluids by placing samples on a rocker or inverting each sample several times. Pour approximately 0.2 mL of calibrator, control, blood or other biological fluid into a clean test tube or sample cup (this initial step enables visualization of any clots and prevents possible contamination of the original sample by the internal standard solution).

7.6.3 Place the dilutor delivery tip into the specimen, making sure its tip is below the surface of the sample. Activate the dilutor. At this point, the dilutor draws 50 µL of sample into its delivery tube.

7.6.4 Direct the delivery tip into the appropriately labeled headspace vial and activate the dilutor. The dilutor will dispense the specimen and 450 µL of IS solution into the vial.

7.6.5 Flush the dilutor tube as necessary by activating the dilutor one or more times or rinsing with dH_2O, depending on the viscosity or other nature of the specimen. Dispense washings into a waste beaker. Wipe withdraw tip with Kimwipe/tissue paper as needed between sampling and dispensing.

7.6.6 Stopper the headspace vial and place in the sample rack.

7.6.7 Repeat steps 7.6.1 – 7.6.6 for all calibrators, controls and specimens.

7.6.8 NOTE: If analyzing tissue, weigh approximately 0.5 g of tissue and transfer to a headspace vial. Record the weight of the tissue. Pipet 4.5 mL internal standard into the headspace vial. Stopper the headspace vial with the Teflon seal and place in the sample rack.

7.6.9 NOTE: If analyzing tissues as homogenates, weigh approximately 0.5 g of tissue on a balance. Record weight. Place tissue in homogenizer tube and add 4.5 mL of internal standard solution. Homogenize. Transfer 0.5 mL into a headspace vial and stopper with the Teflon or Butyl seal and place in the sample rack.

7.6.10 Seal all headspace vials by crimping the aluminum rings over the Teflon seals.

7.6.11 Load headspace vials in the headspace auto sampler.

7.7 Headspace Analysis

7.7.1 Gas Chromatograph Operational Parameters. The following conditions are recommended starting parameters. Instrument conditions may be adjusted to permit improved performance.

- Oven 40ºC Isothermal
- Injector 200ºC
- Detectors (FID) 250ºC
  - Hydrogen flow 30 mL/min
  - Air flow 400 mL/min
  - Make-up flow 25 mL/min or 45 mL/min
  - Make-up gas helium or nitrogen
- Inlet
  - Split
  - Split ratio 10:1
  - Split flow 70 mL/min
  - Total flow 80 mL/min
  - Pressure 24 psi constant pressure mode
  - Temperature 110˚C
  - Septum purge flow 3 mL/min

7.7.2 Headspace Sampler Operational Parameters. The following conditions are recommended starting parameters. Autosampler parameters may be adjusted to permit improved performance.

- Sample Oven 70ºC
- Sample Valve 80ºC
- Transfer Line 90ºC
- GC Cycle 4.5 min
- Vial Equilibration 7.0 min
- Injection Duration 1.0 min
- Vial shaking Off
- Fill mode Default
- Loop Fill Pressure 15 psi
- Loop Fill Mode Custom
- Loop Ramp Rate 30 psi/min
- Loop Final Pressure 1.5 psi
- Loop Equilibration Time 0.05 min
- Extraction Mode Single
- Vent After Extraction ON
- Post Injection Purge 200 mL/min for 3 min

7.7.3 Daily Calibration (Pre-run). The method is calibrated prior to each day's batch sample analysis. Analyze the calibrators, negative control, NIST traceable 0.08% w/v ethanol control and NIST traceable or in-house multicomponent alcohol control on the pre-run batch. Calibrate the method. If the calibrators and/or controls do not satisfy quality control criteria, rerun the affected calibrator or control. If, after data reprocessing, the calibrators and/or controls still do not satisfy quality control criteria, then appropriate measures must be taken to rectify the problem (instrument maintenance, open or prepare new calibrators or controls, etc). Document such actions and measures in the BAC instrument log.

7.7.4 A STAT case sample(s) may be analyzed within a pre-run sequence provided it is bracketed with acceptable calibrators and controls and that appropriate QA/QC measures are applied. Appropriate measures include a vial verification performed by an independent analyst; a documented review of all calibrators, controls and case samples by an independent analyst; and documentation of chain of custody and aliquots. Document this STAT exception, review and communication with the customer on an MFR in the case file.

7.7.5 Batch sample analysis. Headspace alcohol analysis is performed as a batch analysis. Analyze one control at the beginning of the batch (if the pre-run is not immediately prior to the start of the batch) and, at a minimum, after every 10 injections.With smaller batch sizes, controls may be run more often, for example after every 5 samples. The ethanol controls are: 0.00%, 0.05%, 0.10%, 0.20%, and 0.30% by weight by volume. All control levels shall be run within the batch.

7.7.6 Vial Verification. After the completion of the batch, the identity of each vial in the headspace sampler is verified with the sequence table and the Alcohol Batch Worksheet. Vial verification is performed by an analyst other than the operator and is documented by initials and date on the printed sequence table.

7.8 Calculation

7.8.1 Volatiles are identified based on relative retention times compared to calibrators for both columns. Identification is performed by instrument software. Retention times for both analyte and internal standard must be within ± 2% of the retention time obtained from the average of the calibrators.

7.8.2 Concentration is calculated automatically by the software based on linear regression of the 5 point calibration curve (3 or 4 points for methanol, acetone and isopropanol) based on peak area or peak height measurement. The data from the Rtx-BAC1 or Agilent DB-ALC1 or Agilent DB-BAC1 UI column is utilized for quantitative results.

7.8.3 Tissue concentration is calculated as follows:

$$\text{Chromatogram concentration} \times \frac{0.5\ \text{g}}{\text{weighed amount}} = \text{volatile tissue concentration \% (w/w)}$$

7.9 Quality Control

7.9.1 Daily Calibration (Pre-Run). Acceptable tolerance for ethanol calibrators is ± 6% of the target concentration or 0.0040% w/v, whichever is greater. Acceptable tolerance for methanol, acetone and isopropanol is ± 10% of the target concentration or 0.0050% w/v, whichever is greater. Samples may not be analyzed prior to an acceptable pre-run for a particular analyte.

7.9.2 Negative control. The negative control is injected immediately after the 0.50% calibrator within the pre-run and is used to check for carryover. An acceptable negative control may not contain ethanol greater than 0.0020% w/v. If unacceptable, prepare a fresh negative control. Reinject the 0.50% calibrator followed by the new negative control. If ethanol is still present, perform instrument maintenance to correct the problem and document actions in the BAC instrument log. A negative control is also run within each batch of samples.

7.9.3 Positive controls. Acceptable tolerance for ethanol controls is ± 6% of the target concentration or 0.0040% w/v, whichever is greater. Acceptable tolerance for methanol, acetone and isopropanol controls is ± 10% of the target concentration or 0.0050% w/v, whichever is greater.

7.9.4 Case samples shall be bracketed by acceptable controls. If one control fails, repeat all positive case samples not bracketed between acceptable controls. If more than one control fails, all positive samples in the batch must be repeated. Negative results may be reported. Document corrective actions and exceptions on the Alcohol QC Worksheet and the BAC control chart or BAC instrument log. In general, corrective actions for failed controls may include repeating the batch, recalibrating the instrument, opening new controls or making new calibrators.

7.9.5 At least one control within the batch must be a multi-component alcohol control containing 0.05% w/v acetone, methanol and isopropanol in order to report any methanol, acetone or isopropanol positive cases.

7.9.5.1 When a new multicomponent alcohol control is prepared, analyze the new control against NIST traceable calibrators. The control must be within ± 10% of the target concentration or ± 0.0050% w/v, whichever is greater.

7.9.6 If the specified NIST traceable controls (¶ 7.3.8, last bullet) are temporarily not available (e.g., backorder), alternative concentrations may be substituted (e.g., substitute 0.08% w/v control for 0.10% w/v control) provided there is documentation, explanation and justification in all affected case files. If there is a systemic problem obtaining external controls, the Program Manager shall be notified such that an alternative supplier can be identified and appropriate changes made to this procedure.

7.9.7 Correlation of determination (r^2). The r^2 value for the linear regression curve must be 0.995 or greater. The correlation of determination is automatically printed on the calibration curves and tables.

7.9.8 Replicate tolerance. A minimum of two analyses are required to report a positive volatile. Positive single fluid/tissue cases must be analyzed on two separate batch runs. On cases that have multiple fluids/tissues, the second fluid/tissue may be analyzed on the same or separate batches (see ¶ 2.2.4.3). Determine the average and ± 5% range of replicates if the samples appear to be outside of this tolerance (this only needs to be shown when necessary to demonstrate a sample outside of the ± 5% range).

7.9.8.1 Replicates must be within the ± 5% range or within ± 0.0040% (w/v) of the mean, whichever is greater. Reanalyze the sample if it is outside of tolerance. Alternatively, the result may be reported qualitatively.

7.9.8.2 An exception to exclude one volatile replicate from the mean ± 5% or ± 0.0040% range may be made if that value causes the mean and ± 5% or ± 0.0040% range to be unacceptable. Document the exception on an MFR in the case file.

7.9.9 New calibrator certification.

7.9.9.1 NIST traceable (external) calibrators will be verified with an acceptable pre-run prior to the analysis of case samples.

7.9.9.2 In-house calibrators: When new calibrators are prepared, analyze the new calibrators and recalibrate the instrument. Run the NIST traceable 0.05, 0.08, 0.10, 0.20 and 0.30% w/v ethanol controls and the NIST traceable Multicomponent Alcohol controls to certify the new calibrators.

7.9.9.2.1 The new ethanol calibrators and controls must be within ± 6% of their target concentrations or 0.0040% w/v, whichever is greater.

7.9.9.2.2 Acetone, methanol and isopropanol controls must be within ± 10% of the target concentration or 0.0050% w/v, whichever is greater.

7.9.10 Review. After the completion of a batch analysis, the entire run of samples (including calibrators, controls and case samples) shall be reviewed by an independent analyst and this review is documented with a signature on the Alcohol Batch Worksheet.

7.9.11 The batch summary raw data pack shall include the following:

7.9.11.1 Alcohol Batch Worksheet documenting aliquots, analyst, date, independent analyst reviw and DFS case file location of raw data pack.

7.9.11.2 ChemStation pre run sequence

7.9.11.3 ChemStation case sample and control sequence, including the vial check

7.9.11.4 Calibration tables and curves

7.9.11.5 Chromatograms of all calibrators and controls

7.9.11.6 Instrument parameters

7.9.11.7 Alcohol QC Worksheet

7.9.12 Each case file shall contain case sample chromatograms and a copy of the Alcohol Batch Worksheet and Alcohol QC Worksheet. These document aliquots, a summary of QA/QC results, the independent analyst review and DFS case file location of the raw data pack.

7.10 Reporting

7.10.1 For biological fluids, report the volatile concentration of the average of the replicates from the Rtx®-BAC1 or DB-ALC1 or DB-BAC1 UI column data, rounded to three (3) decimal places in % by weight by volume.

7.10.2 For biological tissues, report the volatile concentration of the average of the replicates from the Rtx®-BAC1 or DB-ALC1 or DB-BAC1 UI column data, rounded to three (3) decimal places in % by weight by weight.

7.10.3 Concentrations of ethanol, methanol, isopropanol and acetone less than 0.01% w/v shall be reported as "None Detected."

7.10.4 Negative results may be reported from a single quantitative run.

7.10.5 The upper limit of quantitation (ULOQ) for ethanol is 0.50% w/v. Any ethanol results greater than 0.50% should be repeated using a 1:2 or 1:3 (or other as necessary) dilution with water.

7.10.6 Methanol, isopropanol or acetone concentrations ≥ 0.01% may be reported as “present” (in contaminated or embalmed cases).

7.10.7 Analyze two different biological fluids in postmortem cases (e.g., blood and vitreous or blood and urine) with positive results, provided the samples have been submitted and there is sufficient sample for the analysis. Document exceptions in case file, communication with the customer on an MFR or on the Certificate of Analysis (e.g., “quantity insufficient for analysis”).

7.11 Note

This method may also be used on non-biological samples (e.g., breath alcohol simulator solutions, commercial products, and unknown liquids)

7.12 References

7.12.1 L. C. Nickolls, "A Modified Cavett Method for the Determination of Alcohol in Body Fluids," Nov. 1960, Analyst, Vol. 85, pp 840-942.

7.12.2 B. Kolb, "Head Space Analysis by Means of the Automated Gas Chromatograph F-40 Mulitfract", Bodenseewerk Perkin-Elmer and Co., Technical Manual #15E.

7.12.3 K. M. Dubowski, "Manual for Analysis of Ethanol in Biological Liquids," Department of Transportation Report No. DOT TSC NHTSA-76-4, Jan 1977.

7.12.4 G. Machata, "Determination of Alcohol in Blood by Gas Chromatographic Head Space Analysis," Clin Chem. Newsletter, 4(1972), 29.

7.12.5 7.12.5B.L. Levine, Principles of Forensic Toxicology (Third Edition), American Association for Clinical Chemistry, Inc., 2010, pp 175-190.

7.12.6 Restek Corporation, “Rtx®-BAC1 and BAC2 Columns”, #121-01[001], Revision Date: 5/01.

8 DRUG SCREENING BY ELISA

8.1 Summary

The Immunalysis Direct ELISA Kits are specific and sensitive in-vitro tests to detect the presence of drugs in forensic samples such as whole blood, serum, urine, vitreous and tissue homogenates. The Immunalysis Direct ELISA kits consist of microplates that are coated with a polyclonal antibody with high affinity for the target analytes. These antibodies display cross-reactivity with related drugs within a drug class. An aliquot of the diluted unknown specimen is incubated with a dilution of enzyme-labeled drug derivative in microplate wells coated with fixed amounts of oriented high affinity purified polyclonal antibody. A competitive binding for the antibody binding sites occurs between the enzyme-labeled drug and the drug in the forensic sample. The wells are washed thoroughly to remove any unbound sample or residual reagent and a chromogenic substrate is added. The color produced is stopped using a dilute acid stop solution and the wells are read at 450 nm. The intensity of the color developed is inversely proportional to the concentration of the drug in the sample. The results obtained are presumptive, meaning that any positive result requires appropriate confirmation by a more specific analytical technique such as GC-MS.

8.2 Specimen Requirements

Approximately 50 µL of whole blood, biological fluid(s) or tissue dilutions/homogenates.

8.3 Reagents and Standards

- Immunalysis Direct ELISA Kits for Amphetamine, Acetaminophen, Barbiturates, Benzodiazepines Benzoylecgonine (Cocaine Metabolite), Buprenorphine, Carisoprodol, Fentanyl, Methadone, Methamphetamine, Opiates, Oxycodone/Oxymorphone, PCP, Salicylate, THC Carboxylic Acid, Zolpidem, Dextromethorphan, Diphenhydramine, Tramadol, and Tricyclic Antidepressants. Each kit contains:
    - 96 well microplates coated with polyclonal antibodies. The plates are sealed in a moisture and air barrier pouch with a desiccant. Plates and unused wells should be stored in this pouch at room temperature and are stable for at least as long as the indicated expiration date.
    - Drug conjugate containing drug derivative labeled with horseradish peroxidase in a buffered protein solution with stabilizers containing azide free preservatives. The conjugate should be stored at 2-8°C and is stable until at least the expiration date.
    - TMB chromogenic substrate containing 3, 3', 5, 5' tetramethylbenzidine and urea peroxidase in buffer. The substrate is light sensitive and care should be taken to minimize its exposure to light. It should be stored at 2-8°C and is stable until at least the expiration date.
    - Stop reagent, 1N hydrochloric acid. It should be stored at 2-8°C and is stable until at least the expiration date.
    - Kit insert containing manufacturer-provided instructions and information.
    - Note: Immunalysis kits should be tracked by kit lot number. The conjugates and plates are lot number specific and must be matched with each other. TMB and stop solution are not lot number specific. Kits with identical lot numbers may be combined. Upon receipt in the laboratory, the kits should be opened and components labeled and separated for proper storage. The kit and component lot numbers should be recorded.
- Acetaminophen (APAP) powder
- Amphetamine, 1 mg/mL
- Butalbital, 1 mg/mL
- Buprenorphine, 100 µg/mL
- Clonazepam, 1 mg/mL
- Meprobamate, 1 mg/mL

- Benzoylecgonine, 1 mg/mL
- Fentanyl, 100 µg/mL
- Methadone, 1 mg/mL
- Methamphetamine, 1 mg/mL
- Morphine, 1 mg/mL
- Oxymorphone, 1 mg/mL
- Phencyclidine, 1 mg/mL
- Acetylsalicylic Acid (ASA) powder
- 9-Carboxy-11-nor-delta 9-THC (THC-COOH), 1 mg/mL
- Zolpidem, 1 mg/mL
- Dextromethorphan, 1 mg/mL
- Diphenhydramine, 1 mg/mL
- Tramadol, 1 mg/mL
- Nortriptyline, 1 mg/mL
- UTAK Drugs of Abuse Plus Level 1 Control – Negative Control
- UTAK Drugs of Abuse Plus Level 2 Control – Low Positive Control
- UTAK Drugs of Abuse Plus Level 3 Control – Positive Control
- UTAK Drugs of Abuse Plus Level 4 Control – High Positive Control

8.4 Solutions, Standards, Calibrators and Controls

8.4.1 External Calibrators and Controls

Whole blood calibrators/controls may be utilized from UTAK Laboratories which serve as the negative control (NC), low positive control (LPC), positive control (PC) and high positive control (HPC).

8.4.1.1 These are aliquotted, stored at -10 to -20°C and are stable until at least the expiration date. Once thawed, they must be used within 25 days.

8.4.1.2 Analyte concentrations for externally prepared controls:

Analyte	Level 1 – NC (mg/L)	Level 2 - LPC (mg/L)	Level 3 - PC (mg/L)	Level 4 - HPC (mg/L)
Acetaminophen	0	10	20	200
Amphetamine	0	0.025	0.05	0.5
Methamphetamine	0	0.025	0.05	0.5
Benzoylecgonine	0	0.025	0.05	0.5
Butalbital	0	0.5	1	10
Clonazepam	0	0.02	0.04	0.4
Fentanyl	0	0.001	0.002	0.02
Meprobamate	0	2	4	40
Methadone	0	0.025	0.05	0.5
Morphine	0	0.02	0.04	0.4
Oxymorphone	0	0.02	0.04	0.4
Phencyclidine	0	.005	0.01	0.1
Salicylic Acid	0	25	50	500
THC-COOH	0	0.01	0.02	0.2
Zolpidem	0	0.025	0.05	0.5
Dextromethorphan	0	0.025	0.05	0.5
Diphenhydramine	0	0.05	0.1	1
Tramadol	0	0.125	0.25	2.5
Nortriptyline	0	0.025	0.05	0.5
Buprenorphine	0	0.001	0.002	0.020

8.4.2 Internal Calibrators and Controls

If external calibrators and controls are not available, they may be prepared in-house.

8.4.2.1 Blank blood negative control (**NC**). Blood bank blood previously determined not to contain drugs. Do not use blank blood containing azide as it may affect the ELISA assays.

8.4.2.2 Cutoff Reference Solutions:

8.4.2.2.1 Drugs of Abuse Cutoff Reference solution is prepared in one laboratory, assigned a lot number and distributed to all 4 DFS laboratories. A copy of the preparation records shall be distributed to each laboratory with the solution.

8.4.2.2.2 Add the following volumes to a 100 mL volumetric flask and qs to volume with methanol.

Drug	µL of 1 mg/mL standard	Final concentration (mg/L)
Amphetamine	50	0.5
Benzoylecgonine	50	0.5
Methadone	50	0.5
Methamphetamine	50	0.5
Zolpidem	50	0.5
Clonazepam	40	0.4
Morphine	40	0.4
Oxymorphone	40	0.4
THC-COOH	20	0.2
PCP	10	0.1
Butalbital	1000	10
Meprobamate	4000	40
Fentanyl	20 (100 µg/mL std)	0.02
Buprenorphine	20 (100 µg /mL std)	0.02
Dextromethorphan	50	0.5
Diphenhydramine	100	1.0
Tramadol	250	2.5
Nortriptyline	50	0.5

8.4.2.2.3 Acetaminophen/Salicylate Cutoff Reference Solution, 0.2 mg/mL and 0.5 mg/mL respectively: Weigh 20 mg acetaminophen and 50 mg acetylsalicylic acid. Transfer to a 100 mL volumetric flask and qs to volume with methanol.

8.4.2.2.3.1 This solution is prepared in one laboratory, assigned a lot number and distributed to all 4 DFS laboratories. A copy of the preparation records shall be distributed to each laboratory with the solution.

8.4.2.2.4 The cutoff reference solutions are stable in excess of 24 months when stored at -10 to -20°C.

8.4.2.3 Blood Positive Control (PC)

8.4.2.3.1 Prepare spiked blood PC by adding 100 µL of Drugs of Abuse Cutoff Reference Solution and 100 µL of Salicylate/Acetaminophen Cutoff Reference Solution (as needed) to appropriately labeled tube. Dry tube under nitrogen to evaporate methanol. Reconstitute in 1 mL blank blood to prepare the following drug cutoff

concentrations (mg/L). Vortex briefly. Once prepared, store at 2-8°C and use within 30 days.

Drug	Final Cutoff Concentration (mg/L)
Amphetamine	0.05
Benzoylecgonine	0.05
Methadone	0.05
Methamphetamine	0.05
Zolpidem	0.05
Clonazepam	0.04
Morphine	0.04
Oxymorphone	0.04
THC-COOH	0.02
PCP	0.01
Butalbital	1
Meprobamate	4
Fentanyl	0.002
Acetaminophen	20
Salicylate	50
Buprenorphine	0.002
Dextromethorphan	0.05
Diphenhydramine	0.10
Tramadol	0.25
Nortriptyline	0.05

8.4.2.4 Blood Low Positive Control (LPC)

8.4.2.4.1 Prepare spiked blood LPC by adding 50 µL of Drugs of Abuse Cutoff Reference Solution and 50 µL of Salicylate/Acetaminophen Cutoff Reference Solution (as needed) to appropriately labeled tube. Dry tube under nitrogen to evaporate methanol. Reconstitute in 1 mL blank blood. Vortex briefly. Once prepared, store at 2-8°C and use within 30 days. Final concentrations will be ½ the values listed in 8.4.2.3.1

8.4.2.5 Blood High Positive Control (HPC)

8.4.2.5.1 Prepare spiked blood HPC by adding 1 mL of Drugs of Abuse Cutoff Reference Solution and 1 mL of Salicylate/Acetaminophen Cutoff Reference Solution (as needed) to appropriately labeled tube. Dry tube under nitrogen to evaporate methanol. Reconstitute in 1 mL blank blood. Vortex briefly. Once prepared, store at 2-8°C and use within 30 days. Final concentrations will be ten times the values listed in 8.4.2.3.1

8.5 Apparatus

8.5.1 Test tubes

8.5.2 Screw cap test tubes, 16 x 100 mm disposable glass (for drug conjugates)

8.5.3 Vortex mixer

8.5.4 Micropipets, 8 channel multichannel pipet, pipet tips

8.5.5 Timer

8.5.6 Automated liquid-handling robot (e.g., TECAN MiniPrep or TECAN EVO)

8.5.7 Micro-plate washer

8.5.8 Micro-plate Reader

8.5.9 Computer/printer with Magellan, TECAN and Access software

8.5.9.1 Drug Screening Panels (additional analyses may be added to any panel):

8.5.9.1.1 DUID Panel

Amphetamine
Barbiturates
Benzodiazepines
Buprenorphine
Carisoprodol
Cocaine metabolite
Fentanyl
Methadone
Methamphetamine/MDMA
Opiates
Oxycodone/Oxymorphone
PCP
THC-COOH
Zolpidem
Dextromethorphan
Diphenhydramine
Tramadol
Tricyclic Antidepressants

8.5.9.1.2 Abused Drug Panel

Cocaine metabolite
Opiates
Oxycodone/Oxymorphone
Methamphetamine/MDMA
PCP
Methadone
Fentanyl

8.5.9.1.3 Postmortem Tox panel

Barbiturates
Benzodiazepines
Buprenorphine
Carisoprodol
Cocaine metabolite
Fentanyl
Methadone
Methamphetamine/MDMA
Opiates
Oxycodone/Oxymorphone
PCP
Zolpidem

8.5.9.1.4 X (extra) panel

Amphetamine
Acetaminophen
Acetylsalicylic acid (salicylate)

8.5.9.1.5 Any analyte may be run individually by request or as needed

8.5.9.2 Assays, cutoffs and volumes of diluted samples for each assay

Assay	Cutoff (mg/L)	Sample Volume (µL)	Conjugate Volume (µL)
Acetaminophen	20	10	100
Amphetamine	0.05	10	100
Barbiturates	1	10	100
Benzodiazepines	0.04	40	100
Buprenorphine	0.002	100	100
Carisoprodol	4	10	100
Cocaine metabolite	0.05	20	100
Fentanyl	0.002	100	100
Methadone	0.05	20	100
Methamphetamine/MDMA	0.05	10	100
Opiates B	0.04	20	100
Oxycodone/Oxymorphone	0.04	10	100
PCP	0.01	20	100
Salicylate	50	10	100
THC-COOH	0.02	20	100
Zolpidem	0.05	20	100
Dextromethorphan	0.05	40	50
Diphenhydramine	0.10	40	50
Tramadol	0.25	75	50
Tricyclic Antidepressants	0.05	50	50

8.6 Procedure

8.6.1 Allow all biological samples and reagents come to room temperature before starting procedure.

8.6.2 Label glass disposable test tubes: NC, LPC, PC, HPC and case sample IDs. Prepare controls per ¶ 8.4.

8.6.3 Briefly mix each sample. Pour off approximately 100 µL sample into a clean test tube (this initial step enables visualization of any clots and prevents possible cross contamination of samples with micropipette or diluter).

8.6.4 Dilute each sample 1:20 by mixing 50 µL of sample with 950 µL dH_2O in the appropriately labeled tubes, vortex briefly, and centrifuge if necessary. Depending on the quality or quantity of sample, other volumes may be used to achieve similar 1:20 dilutions.

8.6.5 The first four samples of each assay include the NC, LPC, PC and HPC which verify that all reagents and instruments are working properly prior to the analysis of case samples. A second HPC must also be run at the end of a batch to bracket case samples and ensure reagent and instrument reliability throughout the run. Important: no two samples or controls can have the same name. Two positive controls must have unique names, e.g., HPC-1 and HPC-2.

8.6.6 Place all diluted specimens in the appropriate location on TECAN microplate robot. Check samples for bubbles. If any bubbles are present, they must be minimized with a stick or pipet tip before the diluted samples are pipetted into the plates.

8.6.7 Create a sample dilution rack with unique ID name. Enter all sample ID's in the appropriate position with unique DFS forensic number and item number (as needed). Print sample rack list for vial verification. With every batch of samples run on TECAN robot, the identity of each diluted specimen tube is verified with the sample rack list and dilution rack location by an individual other than the operator. Vial verification is documented by initials on the sample rack list.

8.6.8 Fill 16 x 100 mm screw cap tubes with drug conjugates and place on TECAN robot. Conjugate and plate lot numbers must always be matched.

8.6.9 Add microplates containing strips with the corresponding assays to be run. Always fill the rows (with blank wells if necessary). When handling microplates, use caution not to touch the bottom of the microplate as this may interfere with the measurement of absorbance.

8.6.10 Start TECAN microplate robot to select method, sample dilution rack and destination plates (see TECAN operations manual for details).

8.6.11 Once TECAN robot has completed all sample and conjugate additions, remove microplates from the robot.

8.6.12 Incubate microplates for 1-3 hours at room temperature to allow competitive binding to occur.

8.6.13 Wash microplate wells 6 times with dH_2O using the microplate washer.

8.6.14 Invert plates and slap dry on absorbent paper to ensure all residual moisture is removed.

8.6.15 Using an 8 channel multichannel pipet, manually add 100 µL substrate to each well. Add substrate to plates in sequential order.

8.6.16 Incubate plates for approximately 15-60 minutes at room temperature in the dark until appropriate color development is achieved.

8.6.17 In the same sequential order as above, add 100 µL stop solution to each well. This will change blue color to yellow.

8.6.18 Using a dual wavelength plate reader, read absorbance of each plate within 1 hour of yellow color development (See Operations Manual for details).

8.6.19 Print ELISA drug screening sample report for each case file.

8.7 Calculation

8.7.1 The ratio of the absorbances of the positive controls and samples (B) relative to the negative control (B_0) are multiplied by 100 to generate B/B_0 values.

8.7.1.1 If the sample B/B_0 is equal to or less than the B/B_0 of the PC, the sample is presumptive positive for that class of drugs and the result is listed as "pending" for confirmation.

8.7.1.2 If the sample B/B_0 falls between the LPC and PC, the sample could contain low concentrations of drugs. The result is listed as "review" such that the toxicologist will decide whether or not to pursue confirmation of the drug depending on case history.

8.7.1.3 If the sample B/B_0 is greater than the B/B_0 of the LPC, drugs were "none detected" in the sample and the result is listed as "ND."

8.8 Quality Control and Reporting

8.8.1 Quality Control Criteria

8.8.1.1 The NC must be negative relative to the PC (NC B/B_0 > LPC > PC). The absolute absorbance of the NC should be greater than 0.80 except for methadone and benzodiazepine. For methadone and benzodiazepine plates, the NC should be greater than 0.60.

8.8.1.2 The LPC must be negative relative to the PC (LPC B/B_0 < NC > PC).

8.8.1.3 Both HPC values must be positive relative to the PC (HPC B/B_0 < PC).

8.8.2 Corrective action for failed quality control

8.8.2.1 If NC is positive or less than 0.80 (or 0.60 for methadone and benzodiazepine plates), repeat all samples.

8.8.2.2 If the LPC is positive or HPC negative, repeat or send to confirmation all case samples with B/B_0 within 20% above the PC.

8.8.2.3 Exceptions to these guidelines must be authorized by the Program Manager and documented in the case file with an MFR.

8.8.3 All positive results are presumptive and must be confirmed by a more specific, selective and quantitative procedure.

8.8.4 Confirmation testing may be ordered even if the ELISA result is negative. For example, some benzodiazepines differ in their cross-reactivity with the benzodiazepine assay; if the benzodiazepine ELISA demonstrates an elevated response relative to the negative control, a benzodiazepine confirmation may be ordered to test for specific benzodiazepines. Any confirmation testing ordered from an elevated response will need two aliquots independent of the ELISA result.

8.8.5 Due to the specificity and cross reactivity of each assay (see Immunalysis Kit Inserts), the following nomenclature is used to report screened drugs and/or drug classes: cocaine/benzoylecgonine, opiates, oxycodone/oxymorphone, methamphetamine/methylenedioxymethamphetamine (MDMA), phencyclidine, barbiturates, benzodiazepines, carisoprodol/meprobamate, methadone, fentanyl, cannabinoids, zolpidem, diphenhydramine/cyclobenzaprine, tramadol, dextromethorphan, tricyclic antidepressants, buprenorphine/norbuprenorphine.

8.8.5.1 The above-mentioned list is not inclusive of all possible screening assays or combinations of assays and can be modified to reflect the assays utilized.

8.8.5.2 Due to the cross reactivity of citalopram with the diphenhydramine assay, pending results from this assay can be used as confirmation.

8.9 Note

For each batch of samples run on the ELISA system, the worksheets and associated QC data are placed in one unique DFS case file that is referenced on each case sample report. The QC data pack must contain the specimen aliquot worksheet and associated technical review by an independent examiner, plate setup containing kit lot numbers and the TECAN sample rack list including vial verification by an independent examiner and their corresponding initials.

8.10 References

8.10.1 Immunalysis ELISA Kit Inserts, Pomona CA.

8.10.2 TECAN Columbus Pro Washer Instruction Manual, 30008658 2004-12.

8.10.3 TECAN Hydroflex Washer Instruction Manual, 30086671, 2013-08.

8.10.4 TECAN Sunrise Absorbance Reader Instruction Manual, 30008746, 2005-02.

8.10.5 TECAN Miniprep Logic Manual, 160013, July 1999.

8.10.6 Freedom EVO75 Operating Manual, 30093968.00.

8.10.7 Evaluation of Immunalysis ELISA Assays for the Detection of Drugs of Abuse in Postmortem Bile and Urine. Patton, Isenschmid, Helpler and Schmidt. SOFT Annual Meeting, Portland, OR 2003.

8.10.8 Evaluation of Immunalysis ELISA Assays for the Detection of Drugs of Abuse in Postmortem Blood. Isenschmid, Patton, Helpler and Schmidt. TIAFT Annual Meeting, Melbourne, Australia 2003.

8.10.9 Validation of the Immunalysis Microplate ELISA for the Detection of Buprenorphine and its Metabolite Norbuprenorphine in Urine. Miller, Torrance and Oliver. JAT 30: 115-119, March 2006.

8.10.10 Validation of the Immunalysis Microplate ELISA for the Detection of Methamphetamine in Hair. Han, Miller, Lee, Park, Lim, Chung, Wylie and Oliver. JAT 30: 380-385, July 2006

8.10.11 Wagner, R. and McLean, L., in-house development and validation for dextromethorphan, diphenhydramine, tramadol, and tricyclic antidepressants.

9 ACID/NEUTRAL/BASE DRUG SCREEN AND QUANTITATION BY GC AND GC-MS

9.1 Summary

Acidic, neutral and basic drugs are extracted from biological fluids or tissues using solid phase extraction (SPE) or liquid-liquid extraction (LLE) followed by instrumental analysis with gas chromatography and/or gas chromatography-mass spectrometry (GC-MS). This procedure may employ several drug mixes, but at least one positive control and one negative control. The procedure may be used to screen for basic, acidic and neutral drugs. Once drugs have been confirmed, the procedure may be used to quantitate drugs provided at least 3 calibrators are used to generate a response curve.

9.2 Specimen Requirements

1-2 mL whole blood, urine, bile, gastric contents, other fluids or tissue homogenates.

9.3 Reagents and Standards

- Ammonium hydroxide
- Glacial Acetic Acid
- Potassium Hydroxide
- Potassium Phosphate
- Ethyl Acetate
- Methanol
- Acetonitrile
- Dichloromethane
- Isopropyl alcohol
- Hexane
- Toluene
- Isoamyl alcohol
- Potassium or sodium phosphate buffer solution concentrate (1 M, pH 6.0, e.g., Fisher)
- Sodium phosphate, monobasic ($NaH_2PO_4 \cdot H_20$)
- Sodium phosphate, dibasic (Na_2HPO_4)
- Chloroform
- Hydrochloric Acid
- Sodium tetraborate decahydrate
- Sodium hydrogen carbonate
- Potassium carbonate
- Sulfuric acid

9.4 Solutions, Internal Standards, Calibrators and Controls

9.4.1 Solutions for Varian SPE Extraction

9.4.1.1 1 M Acetic Acid. Add 5.75 mL of glacial Acetic Acid to a 100 mL volumetric flask half filled with dH_2O and qs to volume with dH_2O. Store at room temperature for up to two years.

9.4.1.2 5.0 M Potassium Hydroxide. Weigh 28 g of potassium hydroxide into a 100 mL beaker containing approximately 70 mL dH_2O. After the potassium hydroxide has dissolved, transfer to 100 mL volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

9.4.1.3 0.1 M Phosphate Buffer, pH 6.0. Weigh 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5.0 M potassium hydroxide while stirring and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Store at room temperature for up to two years.

9.4.1.4 2% Ammonium Hydroxide in Ethyl Acetate. Pipette 2 mL of concentrated ammonium hydroxide into a 100 mL graduated cylinder filled with 98 mL of ethyl acetate. Cap graduated cylinder with a glass stopper, and mix well. Vent cylinder occasionally. PREPARE SOLUTION FRESH DAILY!

9.4.2 Solutions for UCT CleanScreen® SPE Extraction

9.4.2.1 When using UCT CleanScreen® SPE Extraction columns, either sodium or potassium phosphate buffer may be used. However, the same buffer (sodium or potassium) must be used throughout the duration of the procedure.

9.4.2.1.1 0.1 M Potassium Phosphate Buffer, pH 6.0. Weigh 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5 M potassium hydroxide while stirring and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Store at room temperature for up to two years.

OR

9.4.2.1.2 0.1 M Sodium Phosphate Buffer, pH 6.0. Weigh 1.70g Na_2HPO_4 and 12.14g $NaH_2PO_4 \cdot H_2O$ and transfer to a 1 L volumetric flask containing approximately 800 mL dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5 M sodium hydroxide and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Store at room temperature for up to two years.

9.4.2.2 1.0 M Acetic Acid. Add 5.75 mL of glacial Acetic Acid to a 100 mL volumetric flask half filled with dH_2O and qs to volume with dH_2O. Store at room temperature for up to two years.

9.4.2.3 Ethyl acetate/Hexane, 50:50 v/v. Mix 500 mL ethyl acetate with 500 mL hexane. Store at room temperature for up to two years.

9.4.2.4 Dichloromethane/isopropanol/ammonium hydroxide (78:20:2). Mix 78 mL dichloromethane with 20 mL isopropanol. Mix well. In hood, add 2 mL ammonium hydroxide. Mix gently. PREPARE SOLUTION FRESH DAILY!

9.4.3 Solutions for liquid/liquid base extraction

9.4.3.1 Saturated borate buffer solution. Add sodium tetraborate decahydrate to dH_2O until no more dissolves after shaking vigorously. Decant saturated solution into a glass jar equipped with a volumetric dispenser. Store at room temperature for up to two years.

9.4.3.2 Toluene:Hexane:Isoamyl Alcohol (THIA) extraction solvent (78:20:2), v:v:v: Mix toluene (780 mL), hexane (200 mL), and isoamyl alcohol (20 mL). Store at room temperature for up to two years.

9.4.3.3 Sodium Hydrogen Carbonate/Potassium Carbonate (dry 3:2 w/w) Mix 300 g $NaHCO_3$ with 200 g K_2CO_3. Store at room temperature for up to two years.

9.4.3.4 0.5 N Sulfuric Acid: Add 13.8 mL concentrated sulfuric acid to a 1 L volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

9.4.4 Reagents for liquid/liquid acid/neutral extraction

9.4.4.1 1.0 M sodium phosphate buffer (pH 5.5). Weigh 13.8 g sodium phosphate, transfer to a 100

mL volumetric flask and qs to volume with dH_2O. Adjust pH to 5.5 with 5 M ammonium hydroxide. Store at room temperature for up to two years.

9.4.4.2 0.1 N HCl. Pipet 8.3 mL concentrated hydrochloric acid into a 1 L volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

9.4.5 Internal Standard

Prepare internal standards from drug standards. The concentration of the internal standard should be approximately midrange of suspected analyte concentration. Suitable internal standards for basic drugs include Sertis, methapyrilene or mepivicaine. Suitable internal standards for acidic/neutral drugs include phensuximide, tolylbarbital, methaqualone, cyclopal or hexobarbital. Deuterated internal standards may also be used when performing analysis by GC-MS in SIM mode. The concentration of internal standard may vary depending on type of case analyzed (DUID vs. postmortem) and expected analyte concentrations.

9.4.6 Calibrators

9.4.6.1 Refer to the Toxicology Quality Guidelines for quality control criteria.

9.4.6.2 Due the wide variety of analytes and expected drug concentrations, it's not practical to have a single calibration curve for all analytes. However, the following are some suggested examples of how to prepare calibration curves to target low, intermediate and high analyte concentrations.

9.4.6.2.1 Dilute 1 mg/mL drug stock solutions to working stock solutions (typically 2, 10 or 100 µg/mL).

9.4.6.2.2 To prepare a low concentration calibration curve, pipet the following volumes of working stock solutions to appropriately labeled tubes. To eliminate a solvent effect, calibrators may be dried under nitrogen prior to the addition of blank blood. Add 2 mL blank blood to each tube.

0.05 mg/L calibrator	10 µL of 10 µg/mL working solution
0.10 mg/L calibrator	20 µL of 10 µg/mL working solution
0.20 mg/L calibrator	40 µL of 10 µg/mL working solution
0.50 mg/L calibrator	100 µL of 10 µg/mL working solution
1.0 mg/L calibrator	200 µL of 10 µg/mL working solution

9.4.6.2.3 To prepare a mid concentration calibration curve, pipet the following volumes of working stock solutions to appropriately labeled tubes. To eliminate a solvent effect, calibrators may be dried under nitrogen prior to the addition of blank blood. Add 2 mL blank blood to each tube.

0.10 mg/L calibrator	20 µL of 10 µg/mL working solution
0.20 mg/L calibrator	40 µL of 10 µg/mL working solution
0.50 mg/L calibrator	100 µL of 10 µg/mL working solution
1.0 mg/L calibrator	200 µL of 10 µg/mL working solution
2.0 mg/L calibrator	40 µL of 100 µg/mL working solution

4.0 mg/L calibrator 80 µL of 100 µg/mL working solution

6.0 mg/L calibrator 120 µL of 100 µg/mL working solution

9.4.6.2.4 To prepare a high concentration calibration curve (for acidic/neutral drugs), pipet the following volumes of working stock solutions to appropriately labeled tubes. To eliminate a solvent effect, calibrators may be dried under nitrogen prior to the addition of blank blood. Add 1 mL blank blood to each tube.

1.0 mg/L calibrator 10 µL of 100 µg/mL working solution

2.0 mg/L calibrator 20 µL of 100 µg/mL working solution

5.0 mg/L calibrator 50 µL of 100 µg/mL working solution

10 mg/L calibrator 100 µL of 100 µg/mL working solution

20 mg/L calibrator 200 µL of 100 µg/mL working solution

40 mg/L calibrator 400 µL of 100 µg/mL working solution

60 mg/L calibrator 600 µL of 100 µg/mL working solution

9.4.7 Controls

9.4.7.1 Positive controls are prepared to monitor the performance of the assay. These controls may vary depending on type of case (DUID vs. postmortem). The positive control should contain frequently observed drugs at low concentrations to address sensitivity of the assay. In addition, the positive control should contain drugs of various chromatographic retention times (early and late eluting drugs) to ensure the chromatographic conditions are capable of detecting a number of drugs. The positive controls can be prepared in-house or purchased from UTAK.

9.4.7.2 See Toxicology Quality Guidelines.

9.4.7.3 Negative Control. Blood bank blood previously determined not to contain reportable drugs (i.e., most bloods contain nicotine and caffeine but these drugs are not typically reported).

9.5 Apparatus

9.5.1 Agilent GC-FID, GC-MS and/or GC-NPD, ChemStation software, compatible computer & printer

9.5.2 Test tubes, round bottom, screw cap tubes, borosilicate glass with Teflon caps

9.5.3 Test tubes, round bottom tubes, borosilicate glass

9.5.4 Test tubes, glass centrifuge, conical bottom

9.5.5 Test tubes, round bottom, screw cap tubes, borosilicate glass

9.5.6 Centrifuge capable of 2,000 – 3,000 rpm

9.5.7 Varian Bond Elute LRC Certify solid phase extraction (SPE) columns or Cleanscreen® Extraction Cartridges (ZSDAU020) from United Chemical Technologies (200 mg columns)

9.5.8 Solid phase extraction manifold

9.5.9 Vortex mixer

9.5.10 Evaporator/concentrator

9.5.11 GC autosampler vials and inserts

9.5.12 Test tube rotator

9.5.13 GC-NPD and GC-FID parameters. Instrument conditions may be changed to permit improved performance.

9.5.13.1 Oven program.

- Equilibration time: 0.50 minutes
- Initial temp: 110°C
- Initial time: 1 minutes
- Ramp: 10°C/min
- Final Temp: 290°C
- Final Time: 10 minutes
- Run Time: 29 minutes

9.5.13.2 Inlet.

- Mode: Splitless
- Temperature: 270°C
- Constant pressure: 30 psi
- Purge flow: 60 mL/min
- Purge time: 0.75 min
- Total flow: 64.9 mL/min
- Injection volume: 2.0 µL

9.5.13.3 Detector.

- Temperature: 320°C
- Hydrogen flow: 3.0. mL/min
- Air flow: 60 mL/min
- Mode: Constant column + makeup flow
- Combined flow: 10.0 mL/min
- Injection volume: 2.0 µL
- Makeup flow: On

9.5.13.4 Column: HP1 or 5 MS 30 m x 0.25 mm x 0.25 µm.

9.5.14 GC-MS parameters. Instrument conditions may be changed to permit improved performance.

9.5.14.1 Acquisition Mode: Scan (50 – 550 amu)

9.5.14.2 Column: HP 1 or 5 MS 30 m x 0.25 mm x 0.25 µm

9.5.14.3 Detector Temperature: 280°C

9.5.14.4 Basic drug screen.

9.5.14.4.1 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 110°C
- Initial time: 1 minutes
- Ramp: 10°C/min
- Final Temp: 290°C
- Final Time: 9 minutes
- Run Time: 28 minutes

9.5.14.4.2 Inlet

- Mode: Splitless
- Temperature: 270°C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

9.5.14.5 Acidic/neutral drug screen.

9.5.14.5.1 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 120°C
- Initial time: 0 minutes
- Ramp 1: 10°C/min
- Final Temp 1: 260°C
- Final Time 1: 0 minutes
- Ramp 2: 30°C/min
- Final Temp 2: 300°C
- Final Time 2: 2.67 minutes
- Run Time: 18 minutes

9.5.14.5.2 Inlet

- Mode: Splitless
- Temperature: 270°C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

9.6 Procedure

9.6.1 Extraction Option 1, Varian SPE Columns

9.6.1.1 Allow all biological specimens to come to room temperature before starting procedure.

9.6.1.2 Label screw cap tubes accordingly. Prepare calibrators and/or controls.

9.6.1.3 Pipet 2 mL of corresponding negative and positive control bloods and case sample bloods, blank blood, and/or fluids or tissue homogenates in appropriately labeled tubes.

9.6.1.4 Pipet internal standard into all tubes and vortex.

9.6.1.5 Add 6 mL of acetonitrile, cap and immediately shake each tube. Put tubes on mechanical rotator for 10 minutes. Note: urine samples do not require acetonitrile precipitation of proteins (start at step 9.6.1.10).

9.6.1.6 Centrifuge tubes at approximately 2500 rpm for 15 minutes to achieve separation.

9.6.1.7 Decant acetonitrile supernatant into labeled, disposable, borosilicate glass culture tubes.

9.6.1.8 Evaporate acetonitrile to 1-2 mL in an evaporator/concentrator.

9.6.1.9 Add dH_2O to each tube to bring the total volume to approximately 3 mL

9.6.1.10 Add 2 mL of pH 6.0, 0.1 M potassium phosphate buffer to all tubes and vortex.

9.6.1.11 Solid phase extraction (SPE). Place labeled SPE cartridges in the extraction manifold. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

9.6.1.11.1 Condition columns with 2 mL methanol and aspirate.

9.6.1.11.2 Add 2 mL pH 6.0 phosphate buffer and aspirate.

9.6.1.11.3 Pour specimens into appropriate SPE columns. Aspirate slowly so that the sample takes at least 2 minutes to pass through the column.

9.6.1.11.4 Add 1 mL of 1 M acetic acid to each column and aspirate. Dry columns under full vacuum/pressure for at least 5 minutes.

9.6.1.11.5 If only extracting basic drugs, add 6 mL methanol, aspirate under full vacuum/pressure for at least 2 minutes.

9.6.1.11.6 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

9.6.1.11.7 Elute base drugs by adding 2 mL of ammonium hydroxide:ethyl acetate(2:98) to each column. Collect eluate in conical test tubes by column aspiration or gravity drain.

9.6.1.12 Evaporate eluates to dryness at approximately 50°C under nitrogen.

9.6.1.13 Add 50 µL ethyl acetate to basic drug extracts. Vortex and transfer to autosampler vials.

9.6.1.14 Transfer autosampler vials to the GC and/or GC-MS. Drug retention time and GC-MS spectral match are used to identify drugs.

9.6.2 Extraction Option 2, CleanScreen SPE Columns.

9.6.2.1 Allow all biological specimens to come to room temperature before starting procedure.

9.6.2.2 Label clean screw cap tubes accordingly. Prepare calibrators and/or controls.

9.6.2.3 Pipet 2 mL of corresponding negative and positive control bloods and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes.

9.6.2.4 Pipet internal standard(s) into all tubes and vortex.

9.6.2.5 Add 4.0 mL deionized water to each tube. Mix, vortex briefly and let stand for 5 minutes.

9.6.2.6 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Transfer supernatant to clean tubes and discard the tube with the remaining pellet.

9.6.2.7 Add 2.0 mL of pH 6 phosphate buffer, mix and vortex. As necessary adjust the pH to 5.5 to 6.5 with additional 0.1 M phosphate buffer. Note: larger volumes of water or phosphate buffer may be used to further dilute some specimens prior to SPE analysis

9.6.2.8 Solid phase extraction. Place labeled SPE cartridges in the extraction manifold. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

9.6.2.8.1 Add 3 mL hexane to each column and aspirate.

9.6.2.8.2 Add 3 mL methanol to each column and aspirate.

9.6.2.8.3 Add 3 mL dH_2O and aspirate.

9.6.2.8.4 Add 1 mL of 0.1 M pH 6.0 phosphate buffer and aspirate.

9.6.2.8.5 Without delay, pour specimens into appropriate SPE columns. Elute from cartridges under vacuum at approximately 1-2 mL/ minute flow.

9.6.2.8.6 Add 3 mL dH_2O and aspirate at ≤ 3inches of mercury.

9.6.2.8.7 Repeat the dH_2O wash.

9.6.2.8.8 Wash with 2.0 mL 1.0 M acetic acid and aspirate.

9.6.2.8.9 If only extracting basic drugs, add 3 mL methanol, aspirate under full vacuum/pressure for at least 2 minutes and skip to step 9.6.2.8.15.

9.6.2.8.10 If extracting acidic/neutral and basic drugs, dry columns under full vacuum/pressure for at least 2 minutes.

9.6.2.8.11 Add 2 mL hexane and aspirate.

9.6.2.8.12 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

9.6.2.8.13 Elute acid/neutral drugs by adding 3 mL of hexane/ethyl acetate (50:50 v/v) to each column. Collect eluate in conical test tubes by gentle column aspiration or gravity drain.

9.6.2.8.14 Remove acid/neutral conical test tubes. Add an additional 3 mL methanol to all SPE columns and aspirate to waste under full vacuum/pressure.

9.6.2.8.15 Add 2 mL hexane to each column. Dry columns at ≥ 10 inches of mercury for five minutes.

9.6.2.8.16 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

9.6.2.8.17 Elute basic drugs by adding 3 mL of freshly prepared methylene chloride/isopropanol/ammonium hydroxide solution to each column. Collect eluate in conical test tubes by column aspiration or gravity drain.

9.6.2.8.18 Elute at 1 - 2 mL/minute (no vacuum) and collect eluates.

9.6.2.9 Evaporate eluates to dryness at approximately 50°C under nitrogen.

9.6.2.10 Reconstitute the residue with 50-200 µL of toluene/ hexane/isoamyl alcohol

9.6.2.11 Vortex and transfer to autosampler vials.

9.6.2.12 Transfer autosampler vials to the GC and/or GC-MS. Drug retention time and GC-MS spectral match are used to identify drugs.

9.6.3 Extraction Option 3, Basic LLE

9.6.3.1 Allow all biological specimens to come to room temperature before starting procedure.

9.6.3.2 Label clean screw cap tubes accordingly. Prepare calibrators and/or controls.

9.6.3.3 Pipet 2 mL of corresponding negative and positive control bloods and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes.

9.6.3.4 Pipet internal standard into all tubes and vortex.

9.6.3.5 Add 2 mL of saturated borate buffer to each tube.

9.6.3.6 Add 5 mL of toluene/hexane/isoamyl alcohol extraction solvent to each tube.

9.6.3.7 Rotate tubes for 20 minutes.

9.6.3.8 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

9.6.3.9 Transfer the top (organic) layer to appropriately labeled screw-cap test tubes. Discard lower (aqueous) layer.

9.6.3.10 Add 2 mL of 0.5N sulfuric acid to tubes. Cap and rotate 20 minutes. Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

9.6.3.11 Aspirate off top (organic) layer and discard.

9.6.3.12 Adjust aqueous layer to a basic pH by slowly adding solid 3:2 $NaHCO_3/K_2CO_3$ buffer until effervescence ceases. Then add approximately 0.3 g excess $NaHCO_3/K_2CO_3$ buffer to saturate the aqueous layer.

9.6.3.13 Add 200 µL of toluene/ hexane/isoamyl alcohol extraction solvent to each tube, cap tubes and vortex for 10-15 seconds. Centrifuge tubes at approximately 2500 rpm for 15 minutes to achieve separation.

9.6.3.14 Transfer approximately 200 µL of top (organic) layer into GC autosampler vials.

9.6.3.15 Transfer autosampler vials to the GC and/or GC-MS. Drug retention time and GC-MS spectral match are used to identify drugs.

9.6.4 Extraction Option 4, Acid/Neutral LLE

9.6.4.1 Allow all biological specimens to come to room temperature before starting procedure.

9.6.4.2 Label clean screw cap tubes accordingly. Prepare calibrators and/or controls.

9.6.4.3 Pipet 1-2 mL of corresponding negative and positive control bloods and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes.

9.6.4.4 Pipet internal standard into all tubes and vortex.

9.6.4.5 Add 1 mL of pH 5.5 sodium phosphate buffer to each tube.

9.6.4.6 Add 5 mL of ethyl acetate to each tube.

9.6.4.7 Rotate tubes for 20 minutes.

9.6.4.8 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

9.6.4.9 Transfer the top (organic) layer to appropriately labeled screw-cap test tubes.

9.6.4.10 Evaporate to dryness at 50-60°C under nitrogen.

9.6.4.11 Reconstitute each sample with 0.5 mL hexane. Vortex briefly.

9.6.4.12 Add 2 mL 0.1 N HCl to each tube. Vortex for 30 seconds.

9.6.4.13 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

9.6.4.14 Aspirate and discard upper (organic) layer.

9.6.4.15 Add 5 mL chloroform to each tube. Vortex for 30 seconds.

9.6.4.16 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

9.6.4.17 Aspirate and discard upper layer.

9.6.4.18 Evaporate to dryness at 50-60°C under nitrogen.

9.6.4.19 Reconstitute samples with 50 µL ethyl acetate.

9.6.4.20 Vortex briefly and transfer to autosampler vials.

9.6.4.21 Transfer autosampler vials to the GC and/or GC-MS. Drug retention time and GC-MS spectral match are used to identify drugs.

9.7 Calculation

9.7.1 GC-NPD and GC-FID Data.

9.7.1.1 Evaluate positive control to ensure efficiency of extraction and proper operation of the GC-NPD or GC-FID.

9.7.1.2 By comparing GC-NPD or GC-FID retention times to known retention times from retention time tables (i.e., caffeine, nicotine and cotinine) some cases may be determined to be “negative” for drugs. In cases with peaks indicating the presence of drugs other than caffeine, nicotine and cotinine, reinject the extracts with significant findings on the GC-MS for

confirmation. Often, the GC-NPD results may be useful when attempting to confirm drugs by GC-MS.

9.7.1.3 Negative Control. The negative control is used as an interpretative aid in assessing internal standard recovery and identifying "junk" peaks that may be common in all samples.

9.7.1.4 For quantitative analysis, prepare a response curve of area (height) of analyte to area (height) of internal standard ratio versus calibrator concentration. Calculate the analyte concentration by interpolation of the linear plot. It is acknowledged that some assays are inherently non-linear and the use of quadratic models may be necessary and appropriate, and should be verified using low, medium and high controls. The response curve and determined unknown specimen concentration(s) are generated by the instrument software.

9.7.1.5 See Toxicology Quality Guidelines.

9.7.2 GC-MS Data

9.7.2.1 Case samples. Take spectra of significant peaks on the TIC. Include spectra and spectral library matches for identified drugs or suspect compounds (excluding non-reported drugs such as caffeine, nicotine and cotinine). Do not include spectrums of "junk" peaks (e.g., fatty acids, phthalates, hydrocarbons, etc.). If needed, use extracted ion profiles to look for drugs indicated by history or GC-NPD results that are not significant peaks on the TIC. Label identified drugs on the TIC or NPD chromatogram.

9.7.2.2 Negative Control. The negative control is used as an interpretative aid in assessing internal standard recovery and identifying "junk" peaks that may be common in all samples.

9.7.2.3 In order to estimate a drug's concentration during screening, a semi-quantitative one-point calculation can be performed using positive controls. Use the drug-to-internal standard ratio (peak area) of the case sample and positive control ratio and concentration to calculate the case sample's drug concentration. Note: the same internal standard must be used for both mix and case sample.

9.7.2.3.1 Quantitation factor: Divide the drug of interest's area by the internal standard's area to obtain the drug-to-internal standard ratio. Divide the drug's concentration by the drug-to-internal standard ratio. The resulting quotient is the quantitation factor.

9.7.2.3.2 Case Sample. Divide the drug of interest's area by the internal standard's area to obtain the drug-to-internal standard ratio. Multiply this ratio by the appropriate quantitation factor to obtain the approximate drug concentration of drug in the case sample. Semi-quantitative concentrations are used to plan dilutions or expected concentrations for additional drug quantitations and to determine whether a drug quantitation is necessary or toxicologically significant.

9.7.3 See Toxicology Quality Guidelines.

9.8 Quality Control and Reporting

See Toxicology Quality Guidelines.

9.9 References

9.9.1 Varian Bond Elute Certify™ Instruction Manual

9.9.2 T. Soriano, C. Jurado, M. Menendez and M. Repetto, "Improved Solid-Phase Extraction Method for Systematic Toxicological Analysis in Biological Fluids." J. Anal. Toxicol. 2001; March (25): 137-143.

9.9.3 W.H. Anderson and D.C. Fuller, “A Simplified Procedure for the Isolation, Characterization, and Identification of Weak Acid and Neutral Drugs from Whole Blood.” J. Anal. Toxicol. 1987, Sep/Oct (11): 198-204.

9.9.4 Randall Edwards, in-house development.

10 CARBOXYHEMOGLOBIN SATURATION DETERMINATION

10.1 Summary

Quantitation by UV-VIS Spectrophotometry

A dilute hemolysate of blood is treated with sodium dithionite to reduce oxyhemoglobin and/or methemoglobin; carboxyhemoglobin remains unaffected. The absorbance of this solution is scanned 650 nm to 500 nm and measured at 541 nm and 555 nm. The absorbance ratio of A_{541nm} / A_{555nm} is calculated and the percent carboxyhemoglobin is determined from the historical calibration curve.

Confirmation by Palladium Chloride Microdiffusion

Carbon monoxide is liberated from blood by strong acid in a microdiffusion cell and palladium chloride, in the center of the diffusion cell, is reduced to metallic palladium which has a silver appearance. The presence of CO in blood can thus be easily detected by the observation of the appearance of the silver film.

10.2 Specimen Requirements

Quantitation: Approximately 0.5 mL of whole blood

Confirmation: Approximately 2.0 mL of blood or mixed tissue containing sufficient quantity of hemoglobin

10.3 Reagents and Standards

Quantitation

- Ammonium hydroxide (NH_4OH), 0.4%. Pipet 15.9 mL of concentrated NH_4OH into a 1 L volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.
- Sodium dithionite (sodium hydrosulfite)

Confirmation

- Hydrochloric acid, concentrated
- Sulfuric acid, concentrated
- Palladium chloride
- Lead acetate
- Glacial acetic acid
- 0.1N Hydrochloric Acid: Cautiously add 8.3 mL of concentrated HCl to approximately 100 mL of dH_2O in a 1 L volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.
- 10% (3.6 N) Sulfuric Acid: Cautiously add 10 mL of concentrated H_2SO_4 to approximately 70 mL of dH_2O in 100 mL volumetric flask. Cool and qs to volume with dH_2O. Store at room temperature for up to two years.
- 0.005 N Palladium Chloride Reagent: Weigh 0.22 g palladium chloride, transfer into a 250 mL volumetric flask and qs to volume with 0.1 N HCl and let stand overnight. Transfer to a 500 mL volumetric flask and qs to volume with 0.1 N HCl. Store at room temperature for up to two years.
- 10% Lead Acetate-Acetic Acid Solution: Add 10 mL of glacial acetic acid to a 100 mL volumetric flask and qs to volume with dH_2O. Saturate the solution with lead acetate by adding lead acetate until no more dissolves after mixing vigorously. Store at room temperature for up to two years.

10.4 Calibrators, Controls and Internal Standards

IL Test™Multi-4™ CO-Oximeter Controls, Instrumentation Laboratory Company, Lexington, MA, stored at 2-8°C.

10.4.1 A certified reference material is not currently available to establish traceability for the calibrators and CO-oximeter controls.

10.5 Apparatus

Quantitation

10.5.1 1 cm UV-VIS cuvetttes

10.5.2 Shimadzu UV-1800 Spectrophotometer with UVProbe software

10.5.3 Method parameters in Spectrum Mode

- Wavelength range (nm): 650-500
- Scan speed: Fast
- Sampling interval: Auto
- Scan mode: Single (or auto)
- Measuring mode: Absorbance
- Attachments: 6-cell

Confirmation

10.5.4 Conway microdiffusion cells (2 well, with cover)

10.5.5 Sealant or dH_2O

10.6 Procedure

Quantitation

10.6.1 Allow all biological samples and reagents to come to room temperature before starting procedure.

10.6.2 Allow instrument to warm up for approximately one hour.

10.6.3 Negative and positive blood control samples are prepared and analyzed as single, reduced samples. Case samples are prepared and analyzed in triplicate: one unreduced and two reduced samples.

10.6.4 To prepare samples, add approximately one to three drops of case sample to a 1 cm cuvette containing 2-3 mL of 0.4% NH_4OH solution (try to achieve an absorbance maximum between 0.90 and 1.05) and mix by inversion.

10.6.5 To prepare reduced samples, add approximately 75 – 100 mg sodium dithionite to above and mix by inversion.

10.6.6 Prepare a reference sample (blank) by adding approximately 2-3 mL of 0.4% NH_4OH solution and approximately 75 – 100 mg sodium dithionite to a 1 cm cuvette. Place in the reference cell position of the instrument.

10.6.7 Analyze samples

10.6.7.1 Scan the absorbance spectrum from 650 nm to 500 nm of each sample.

10.6.7.2 Use the point pick function of the software to extract the absorbance at 541 nm and 555 nm.

10.6.7.3 Print the absorbance spectrum and results.

Confirmation (may not be performed if Quantitation results are < 10% saturation)

10.6.8 Prepare microdiffusion cell with sealant or dH_2O.

10.6.9 Add 2 mL of $PdCl_2$ reagent to center well of microdiffusion cell.

10.6.10 Add 2 mL of blood to one side of the outer ring.

10.6.11 Add 1 mL of 10% H_2SO_4 to the other side of the outer ring. Quickly cover microdiffusion cell and gently rock/rotate to mix blood with sulfuric acid. Diffuse for approximately one hour.

10.6.12 Record results.

10.7 Calculation

10.7.1 Transfer data to the carboxyhemoglobin worksheet to calculate the ratio of the absorbance at 541nm / 555 nm and determine the percent carboxyhemoglobin for the negative, positive control samples and the case samples from the slope of the calibration curve.

10.7.2 The standard curve was obtained by saturating negative blood with known concentrations of HbCO and plotting the known % carboxyhemoglobin samples (10, 20, 50, 70 and 100% HbCO) versus ΔA. The curve is linear from 10-60% carboxyhemoglobin.

10.8 Quality Control and Reporting

Quantitation

10.8.1 Analyze at least one positive and one negative control with each group of case samples.

10.8.2 The negative control must be less than 10% carboxyhemoglobin saturation. The positive control must fall within established ranges provided by the CO-oximeter manufacturer or within 20% of established target concentrations, whichever is greater. If the negative or positive control does not meet acceptance criteria, all samples must be repeated.

10.8.2.1 Positive controls will be tracked using a statewide spreadsheet. Twelve values (run in at least three laboratories) are required before the mean concentration values may be used for acceptance criteria.

10.8.3 The LOQ for the assay is 10% saturation and the ULOQ is 60% saturation. Results below the LOQ are reported as “carboxyhemoglobin none detected at approximately 10% saturation”. Results between the LOQ and ULOQ shall be reported as “carboxyhemoglobin approximately (whole number)% saturation”. Results greater than the ULOQ are reported as “carboxyhemoglobin greater than approximately 60% saturation”.

10.8.4 Samples of questionable quality may be reported as “unsuitable for analysis”. A distinct minimum should appear in the unreduced hemoglobin spectrum and yield a ratio of A_{min}/A_{max} of about 0.8 or less. The absorbance minimum should be at least 20% lower than either adjacent maxima. A less distinct minimum may indicate that the sample is unsuitable for analysis (insufficient hemoglobin).

10.8.5 A positive (> 10%) carboxyhemoglobin result may only be reported if it is confirmed or consistent with the palladium chloride microdiffusion result. If there are inconsistencies between the UV-VIS and palladium chloride results, repeat the analysis. If, after repeat analysis, inconsistencies still exist, the sample should be reported as “unsuitable for analysis”. This exception should be authorized by a supervisor, group supervisor, or toxicologist and documented in the case file with an MFR.

10.8.6 Weak positive results (greater than 10%) on putrefied, decomposed or deteriorated specimens may be reported as less than a toxicologically significant carboxyhemoglobin concentration (e.g., a 12% CoHb on poor quality specimen, confirmed by palladium chloride microdiffusion, may be reported as carboxyhemoglobin less than approximately 15% saturation). This exception should be authorized by a toxicologist and documented in the case file with an MFR.

Confirmation

10.8.7 A silver colored mirror will form in the center well of the dish in positive samples. Negative samples will appear to be unchanged (clear yellow gold color of the palladium chloride reagent). The intensity of the silver mirror will be directly proportional to the concentration of carbon monoxide in the blood.

10.8.8 A very small but noticeable silver mirror (particles) indicates about 10% saturation.

10.8.9 Record the intensity of the reaction by using "+++" to indicate the strongest reaction (e.g., > 60% saturation), "++" (e.g., 30-50% saturation), "+" (e.g., 10-20% saturation), or "-" (e.g., < 10% saturation).

10.8.10 Analyze at least one level of the positive controls and a negative control with each group of case samples.

10.8.11 A positive (> 10%) carboxyhemoglobin result may only be reported if it is confirmed or consistent with the palladium chloride microdiffusion result. If there are inconsistencies between the Quantitation and Confirmation results, repeat the analysis. If, after repeat analysis, inconsistencies still exist, the sample should be reported as "unsuitable for analysis." This exception should be authorized by a toxicologist, group supervisor, or supervisor and documented in the case file with an MFR.

10.8.12 Weak positive results (> 10%) on putrefied, decomposed, or deteriorated specimens may be reported as less than a toxicologically significant carboxyhemoglobin concentration (e.g., a 12% COHb on poor quality specimen, confirmed by palladium chloride microdiffusion) may be reported as "carboxyhemoglobin less than approximately 15% saturation." This exception should be authorized by a toxicologist, group supervisor, or supervisor and documented in the case file with an MFR.

10.9 Notes

Quantitation

The exact concentration of hemoglobin in NH_4OH is not critical but the resulting absorbance maxima should be approximately 1 A. Ordinarily one to three drops of whole blood per 3 ml NH_4OH should be sufficient. More than four or five drops per 3 ml may indicate that the nature of the sample is questionable. If this occurs, the sample may be reported as "unsuitable for analysis".

Confirmation

Sulfur compounds (e.g., hydrogen sulfide from putrefied specimens) may react with $PdCl_2$. For putrefied speciments, substitute lead acetate for 10% sulfuric acid and allow to diffuse for 4 hours.

10.10 References

10.10.1 Tietz, Norbert W., Ph.D. and Fiereck, A., M.S. Annals of Clinical Laboratory Science, Vol. 3, No. 1 pp. 36-42, 1973

10.10.2 B.L. Levine, Principles of Forensic Toxicology, American Association for Clinical Chemistry, Inc., pp. 330-337, 1999.

10.10.3 Ramieri, A., Jr., Jatlow, P. and Seligson, D. New method for rapid determination of carboxyhemoglobin by use of double-wavelength spectrophotometry (ΔA Method). Clinical Chemistry, Vol. 20, No. 2, 1974.

10.10.4 van Kampen, E.J. and Klouwen, N. Tidschr. Geneeskd, 98, pp. 161-164, 1954.

10.10.5 Holmium Oxide Glass Wavelength Standards. *Journal of Research at the National Institute of Standards and Technology* Vol 112 (6): 303-306, 2007.

10.10.6 Intrinsic Wavelength Standard Absorption Bands in Holmium Oxide Solution for UV-VISible Molecular Absorption Spectrophotometry. *J Phys Chem Ref Data* Vol 34(1): 41-56, 2005.

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11 OPIOID QUANTITATION AND CONFIRMATION BY GC-MS

11.1 Summary

Opioid drugs are extracted from biological samples using an acetonitrile precipitation, solid phase extraction (SPE) and analyzed by selected ion monitoring GC-MS. Morphine, codeine, 6-acetylmorphine, hydrocodone, hydromorphone, oxycodone, oxymorphone, and deuterated internal standards are extracted and analyzed simultaneously. The keto opioids are reacted with hydroxylamine to form oxime derivatives to prevent tautomerism. Hydroxyl groups of all opioids are further derivatized with BSTFA with 1% TMCS to form trimethylsilyl derivatives. The high mass compounds formed enable chromatographic separation and quantitation of all seven opioids simultaneously.

11.2 Specimen Requirements

Two mL blood, urine, vitreous humor, gastric or tissue homogenate

11.3 Standards and Reagents

11.3.1 Standards

- 6-acetylmorphine, 1 mg/mL
- 6-acetylmorphine-d_3, 1 mg/mL or 100 µg/mL
- Codeine, 1 mg/mL
- Codeine-d_3, 1 mg/mL
- Morphine, 1 mg/mL
- Morphine-d_3, 1 mg/mL
- Hydrocodone, 1 mg/mL
- Hydrocodone-d_3, 1 mg/mL or 100 µg/mL
- Hydromorphone, 1 mg/mL
- Hydromorphone-d_3, 1 mg/mL or 100 µg/mL
- Oxycodone, 1 mg/mL
- Oxycodone-d_3 or Oxycodone-d_6, 1 mg/mL
- Oxymorphone, 1 mg/mL
- Oxymorphone-d_3, 1 mg/mL or 100 µg/mL

11.3.2 Reagents

- Ammonium hydroxide
- Acetic acid, glacial
- Hydrochloric Acid, concentrated
- Potassium Hydroxide
- Potassium Phosphate
- BSTFA containing 1% TMCS, stored at 2-8°C
- Ethyl Acetate
- Methanol
- Acetonitrile
- Hydroxylamine Hydrochloride
- Hexane
- Sodium acetate trihydrate
- Methylene chloride
- Isopropanol
- Ammonium hydroxide
- Potassium or sodium phosphate buffer solution concentrate (1 M, pH 6.0, e.g., Fisher)
- Sodium phosphate, monobasic ($NaH_2PO_4 \cdot H_20$)
- Sodium phosphate, dibasic (Na_2HPO_4)

11.4 Solutions, Internal Standards, Calibrators and Controls

11.4.1 Solutions

11.4.1.1 1 M Acetic Acid. Add 5.75 mL of glacial acetic acid to a 100 mL volumetric flask half filled with dH_2O and qs to volume with dH_2O. Store at room temperature for up to two years.

11.4.1.2 5.0 M Potassium Hydroxide. Weigh 28 g of potassium hydroxide into a 100 mL volumetric flask. Dissolve the potassium hydroxide with dH_2O and qs to volume with dH_2O. Store at room temperature for up to two years.

11.4.1.3 0.1 M Phosphate Buffer, pH 6.0. Weigh out 13.61 g of KH_2PO_4 and transfer into a 1 liter volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5.0 M potassium hydroxide while stirring and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Store at room temperature for up to two years.

11.4.1.4 2% Ammonium Hydroxide in Ethyl Acetate. Pipette 2.0 mL of concentrated ammonium hydroxide into a 100 mL graduated cylinder filled with 98 mL of ethyl acetate. Cap graduated cylinder with a glass stopper, and mix well. Vent cylinder occasionally. PREPARE SOLUTION FRESH DAILY!

11.4.1.5 1% Hydroxylamine. Weigh 250 mg of hydroxylamine, add to a 25 mL volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

11.4.1.6 100 mM acetate buffer (pH 4.5). Weigh 2.93 g sodium acetate trihydrate into a 500 mL volumetric flask. Add approximately 400 mL dH_2O to dissolve sodium acetate. Add 1.62 mL glacial acetic acid. Adjust pH to 4.5 with 1 M acetic acid and qs to volume with dH_2O. Store at room temperature for up to two years.

11.4.1.7 Dichloromethane/isopropanol/ammonium hydroxide (78:20:2). Mix 78 mL dichloromethane with 20 mL isopropanol. Mix well. In hood, add 2 mL ammonium hydroxide. Mix gently. PREPARE SOLUTION FRESH DAILY!

11.4.2 Calibrators and internal standards. Calibrators may be prepared in pools and stored as frozen aliquots or as fresh spikes daily.

11.4.2.1 Spiked calibrator preparation.

11.4.2.1.1 Calibrator working stock standard (4.0/1.0 µg/mL). Add 100 µL each of 1 mg/mL codeine, morphine, hydrocodone, hydromorphone, oxycodone and oxymorphone and 25 µL 6-acetylmorphine standards into the same 25 mL volumetric flask and qs to volume with methanol.

11.4.2.1.2 Pipet the following volumes of working stock standard into appropriately labeled tubes 16 x 125 mm screw cap test tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 2 mL blank blood to each tube.

Blood Final Conc (mg/L) Opioid/6AM	Volume (µL) Working Stock Standard (4/1 µg/mL)
0.01/0.0025	5
0.02/0.005	10
0.04/0.010	20
0.10/0.025	50

0.30/0.075	150
0.50/0.125	250
0.80/0.200	400

11.4.2.2 Internal standards. Internal standard spiking stock. Pipet 25 µL of 1 mg/mL opioid deuterated internal standards and 250 µL of 100 µg/mL deuterated internal standards (except 6-acetylmorphine) to a 10 mL volumetric flask. Add 100 µL of 100 µg/mL 6-acetylmorphine-d_3 into the same volumetric flask. qs to volume with methanol. Add 100 µL of internal standard spiking stock to 2 mL blank blood for final concentration of 50 µg/L for 6-acetylmorphine and 125 µg/L for all other deuterated opioids.

11.4.2.3 A single internal standard (listed in 13.3.1) may be used for more than one analyte.

11.4.3 Controls.

11.4.3.1 Negative blood control. Blood bank blood or equivalent previously determined not to contain opioids.

11.4.3.2 Positive blood control (100 µg/L codeine, morphine, hydrocodone, hydromorphone, oxycodone, oxymorphone and 6-acetylmorphine). The positive control may be purchased or prepared in-house. Do not use calibrator working stock standard to prepare controls. The in-house positive control may be prepared as a pooled control or spike. Additional positive control concentrations may be prepared.

11.4.3.2.1 Pooled control working stock standard (5 µg/mL codeine, morphine, hydrocodone, hydromorphone, oxycodone, oxymorphone and 2.5 µg/mL 6-acetylmorphine). Pipet 50 µL each of 1 mg/mL codeine, morphine, hydrocodone, hydromorphone, oxycodone, oxymorphone standards and 25 µL of 1 mg/mL 6-acetylmorphine standard into a 10 mL volumetric flask and qs to volume with dH_2O.

11.4.3.2.2 Pooled blood control. Pipet 1.0 mL of control working stock solution into a 50 mL volumetric flask and qs to volume with blank blood. Once the concentration is verified, aliquot 2 mL of positive blood control into 16 x 125 mm screw top tubes. Store aliquots at less than -10°C for up to 2 years for future use.

11.4.3.2.3 Spiked control. From a separate pipeting, prepare the control spiking stock as per 13.4.2.1. Add 50 µL to a test tube. Evaporate methanol, add 2 mL blank blood and vortex. Different concentrations and preparations of spiked controls are permissible.

11.4.3.2.4 Commercial control. UTAK or other commercial vendors. Verify control concentration before placing in-service.

11.5 Apparatus

11.5.1 Agilent GC-MS, ChemStation software, compatible computer and printer.

11.5.2 Column: DB or HP-1 or HP-5 MS; 30 m x 0.25 mm x 0.25 µm.

11.5.3 Test tubes, screw cap tubes, borosilicate glass with Teflon caps.

11.5.4 Test tubes, round bottom tubes, borosilicate glass.

11.5.5 Test tubes, glass tubes, conical bottom.

11.5.6 Centrifuge capable of 2,000 – 3,000 rpm.

11.5.7 Varian Bond Elute LRC Certify solid phase extraction (SPE) columns or United Chemical Technologies (UCT) SPE columns (ZSDAU020).

11.5.8 Solid phase extraction manifold.

11.5.9 Vortex mixer.

11.5.10 Evaporator/concentrator.

11.5.11 GC autosampler vials and inserts.

11.5.12 GC-MS Instrument conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

11.5.12.1 Oven program

- Equilibration time: 0.50 minutes
- Initial temp: 160°C
- Initial time: 0 minutes
- Ramps:

	Final Temp(°C)	Final Time (minutes)
35°C/min	195	0
5°C/min	240	0
30°C/min	300	2.0

- Run time: 14 minutes

11.5.12.2 Inlet

- Mode: Splitless
- Temperature: 250°C
- Constant pressure: 13.88 psi
- Purge flow: 15.0 mL/min
- Purge time: 2.0 min
- Total flow: 18.9 mL/min
- Injection volume: 1.0 µL

11.5.12.3 MS Detector Temperature

- Transfer Line: 280°C
- Source: 230°C
- Quads: 150°C

11.5.12.4 Solvent delay: 10 minutes.

11.5.12.5 Acquisition mode: SIM (selected ion monitoring)

11.5.12.6 EM voltage: 200-600 over autotune depending on needed sensitivity

11.5.12.7 Dwell time: 40 milli-seconds

11.5.12.8 Ion Group 1

• Codeine	371	343	372
• Codeine d_3	374	346	
• Morphine	429	401	430
• Morphine d_3	432	417	

11.5.12.9 Ion Group 2

• Hydrocodone	386	371	387
• Hydrocodone d_3	389	374	
• 6-Acetylmorphine	399	340	400
• 6-Aceylmorphine d_3	402	343	
• Hydromorphone	355	444	429
• Hydromorphone d_3	447	358	

11.5.12.10 Ion Group 3

• Oxycodone	474	459	475
• Oxycodone d_3	477	462	
• Oxycodone d_6	480	465	
• Oxymorphone	532	517	533
• Oxymorphone d_3	535	520	

11.6 Procedure (Two extraction procedures are presented as Option #1 and Option #2)

11.6.1 Note: Urine specimens may be hydrolyzed to remove glucuronide conjugates prior to extraction using one of the following hydrolysis procedures. A glucuronide positive control should be used to ensure the hydrolysis was effective.

11.6.1.1 Enzyme hydrolysis

Add 5000 Fishman units of β-glucuronidase to each mL of urine. Perform hydrolysis as recommended by the supplier based on the source of β-glucuronidase (e.g., 5000 F units/mL *Patella vulgata* in 100 mM acetate buffer (pH 5.0) hydrolyzed at 65°C for 3 hours).

11.6.1.2 Alkaline hydrolysis

Add 80 µL of 10 N NaOH to 2 mL of each urine specimen. The pH should be greater than 10. Cap, vortex and heat at 60°C for 20 minutes. After cooling, add 50 µL of glacial acetic acid and acetate buffer as necessary to neutralize pH.

11.6.2 Extraction Option #1 (Varian Bond Elute SPE columns)

11.6.2.1 Label screw cap tubes accordingly, negative, calibrators, control (s) and case sample IDs.

11.6.2.2 Pipet 2 mL of each case sample into appropriately labeled tubes.

11.6.2.3 Pipet 100 µL of internal standard spiking stock to all tubes and vortex.

11.6.2.4 Add 6 mL of acetonitrile, cap and immediately shake each tube. Rotate tubes for 10 minutes.

11.6.2.5 Centrifuge tubes at approximately 2500 rpm for 15 minutes to achieve separation.

11.6.2.6 Decant supernatant into labeled tubes.

11.6.2.7 Evaporate acetonitrile to 1-2 mL.

11.6.2.8 Add 2 mL of pH 6.0, 0.1 M phosphate buffer to all tubes.

11.6.2.9 Add 0.5 mL of 1% hydroxylamine to each tube. Vortex briefly.

11.6.2.10 Place parafilm over the tops of tubes and incubate at 60°C for one hour. Allow tubes to cool to room temperature.

11.6.2.11 Solid phase extraction (SPE). Place Varian Bond Elute SPE cartridges in the extraction manifold.

11.6.2.11.1 Add 2 mL methanol and aspirate.

11.6.2.11.2 Add 2 mL pH 6.0 phosphate buffer and aspirate. Important! Do not permit SPE sorbent bed to dry. If necessary, add additional buffer to re-wet.

11.6.2.11.3 Pour specimens into appropriate SPE columns. Aspirate slowly so that the sample takes at least 2 minutes to pass through the column.

11.6.2.11.4 Add 1 mL of 1 M acetic acid to each column and aspirate. Dry columns under full vacuum/pressure for at least 5 minutes.

11.6.2.11.5 Add 6 mL of methanol to each column and aspirate. Dry columns under full vacuum/pressure for at least 2 minutes.

11.6.2.11.6 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

11.6.2.11.7 Add 2 mL of 2% ammonium hydroxide in ethyl acetate to each column. Collect eluant in conical test tubes by column aspiration or gravity drain.

11.6.2.12 Evaporate eluates to dryness at approximately 50-60°C under nitrogen.

11.6.2.13 Add 25 µL ethyl acetate and 25 µL BSTFA with 1% TMCS to each tube. Cap and vortex briefly.

11.6.2.14 Incubate for 20 minutes at 90°C.

11.6.2.15 Allow extracts to cool to room temperature.

11.6.2.16 Transfer derivatized extracts to autosampler vials.

11.6.3 Extraction Option #2 (UCT SPE columns)

11.6.3.1 Label tubes accordingly, calibrators, controls and case sample ID's.

11.6.3.2 Prepare calibrators and controls.

11.6.3.3 Pipet 2 mL of each case sample into appropriately labeled tubes.

11.6.3.4 Pipet 100 µL of internal standard spiking stock to all tubes and vortex.

11.6.3.5 Add 3 mL cold (freezer temperature) acetonitrile dropwise to each tube, while vortexing.

11.6.3.6 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

11.6.3.7 Refrigerate at least 2 hours to achieve separation of the layers, as necessary.

11.6.3.8 Transfer supernatants to clean test tubes.

11.6.3.9 Add 2 mL of pH 6.0, 0.1 M phosphate buffer to tubes.

11.6.3.10 Add 0.5 mL of 1% hydroxylamine to each tube and vortex.

11.6.3.11 Cap tubes and incubate at 60°C for one hour. Allow tubes to cool to room temperature.

11.6.3.12 Solid Phase Extraction (SPE). Place UCT SPE cartridges in the extraction manifold. Important! Do not permit SPE sorbent bed to dry. If necessary, add additional buffer to re-wet.

11.6.3.12.1 Add 3 mL hexane and aspirate.

11.6.3.12.2 Add 3 mL methanol and aspirate.

11.6.3.12.3 Add 3 mL dH_2O and aspirate.

11.6.3.12.4 Add 3 mL pH 6.0 phosphate buffer and aspirate.

11.6.3.12.5 Pour specimens into appropriate SPE columns. Aspirate slowly so that the sample takes at least 2 minutes to pass through the column.

11.6.3.12.6 Add 1 mL of 1 M acetic acid to each column and aspirate.

11.6.3.12.7 Add 3 mL methanol and aspirate.

11.6.3.12.8 Dry columns under full vacuum/pressure (15 psi) for at least 10 minutes.

11.6.3.12.9 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

11.6.3.12.10 Add 3 mL of 2% ammonium hydroxide in ethyl acetate (prepared fresh daily) to each column. Collect eluant in conical test tubes by column aspiration or gravity drain.

11.6.3.13 Evaporate eluates to dryness at approximately 50-60°C under nitrogen.

11.6.3.14 Add 30 µL ethyl acetate and 30 µL BSTFA with 1% TMCS to each tube. Cap and vortex briefly.

11.6.3.15 Incubate for 20 minutes at 90°C.

11.6.3.16 Allow extracts to cool to room temperature.

11.6.3.17 Transfer derivatized extracts to autosampler vials.

11.6.4 Extraction Option #3 (UCT SPE columns)

11.6.4.1 Label tubes accordingly, calibrators, controls and case sample ID's.

11.6.4.2 Prepare calibrators and controls.

11.6.4.3 Pipet 2 mL of each case sample into appropriately labeled tubes.

11.6.4.4 Pipet 100 µL of internal standard spiking stock to all tubes and vortex.

11.6.4.5 Add 3 mL cold (freezer temperature) acetonitrile dropwise to each tube, while vortexing.

11.6.4.6 Centrifuge at approximately 2800 rpm for 15 minutes to achieve separation.

11.6.4.7 Refrigerate at least 2 hours to achieve separation of the layers, as necessary.

11.6.4.8 Transfer supernatants to clean test tubes.

11.6.4.9 Evaporate acetonitrile to 2 mL.

11.6.4.10 Add 2 mL of pH 6.0, 0.1 M phosphate buffer to tubes.

11.6.4.11 Add 0.5 mL of 1% hydroxylamine to each tube and vortex.

11.6.4.12 Place parafilm over the tops of tubes and incubate at 60°C for one hour. Allow tubes to cool to room temperature. Place tubes in refrigerator while preparing SPE columns.

11.6.4.13 Solid Phase Extraction (SPE). Place UCT SPE cartridges in the extraction manifold. Important! Do not permit SPE sorbent bed to dry. If necessary, add additional solvent to re-wet.

11.6.4.13.1 Add 3 mL methanol and aspirate.

11.6.4.13.2 Add 3 mL dH_2O and aspirate.

11.6.4.13.3 Add 3 mL pH 6.0 phosphate buffer and aspirate.

11.6.4.13.4 Pour specimens into appropriate SPE columns. Aspirate slowly so that the sample takes at least 2 minutes to pass through the column.

11.6.4.13.5 Wash columns by adding 2 mL dH_2O and aspirate.

11.6.4.13.6 Add 2 mL 100 mM acetate buffer and aspirate.

11.6.4.13.7 Add 3 mL methanol and aspirate.

11.6.4.13.8 Dry columns under full vacuum/pressure (15 psi) for at least 10 minutes.

11.6.4.13.9 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

11.6.4.13.10 Elute opiates by adding 3 mL 78/20/2 methylene chloride/isopropanol/ammonium hydroxide (prepared fresh daily) to each column. Collect eluant in conical test tubes by column aspiration or gravity drain.

11.6.4.14 Evaporate eluates to dryness at approximately 50-60°C under nitrogen.

11.6.4.15 Add 25 µL ethyl acetate and 25 µL BSTFA with 1% TMCS to each tube. Cap and vortex briefly.

11.6.4.16 Incubate for 20 minutes at 90°C.

11.6.4.17 Allow extracts to cool to room temperature.

11.6.4.18 Transfer derivatized extracts to autosampler vials.

11.7 Calculation

Use ChemStation or MassHunter software to calculate the concentrations by interpolation of a linear plot of the response curve based on peak height (or area) ratios (using the target ions listed under GC-MS conditions) versus calibrator concentration.

11.8 Quality Control and Reporting

11.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte. The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

11.8.2 For 6-acetylmorphine, the LOQ is generally 0.005 mg/L.

11.8.2.1 6-acetylmorphine results shall be reported as "present" only.

11.8.2.2 6-acetylmorphine may only be reported if the case specimen 6-AM concentration is at or above the LOD AND morphine is present at or above 0.01 mg/L.

11.8.3 See Toxicology Quality Guidelines

11.9 References

11.9.1 C.W. Jones, G. Chaney and S. Mastorides, "Simultaneous Analysis of Opiates in Urine by SPE and GC-MS with Stabilization of Keto-Opiates via Conversion to Oxime Derivatives." Abstract #28, 1996 SOFT Annual Meeting, Denver, CO, ToxTalk Supplement, December 1996: 20(4): 9.

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11.9.5 G.M. Vallaro and F. Mazur. "Confirmation of Opioids by GC-MS," UMASS Drug Concentration Laboratory SOP.

11.9.6 Varian Bond Elute Certify™ Instruction Manual

12 BARBITURATE AND ACID DRUG QUANTITATION AND CONFIRMATION BY GC AND GC-MS

12.1 Summary

Biological samples are made acidic with monosodium phosphate buffer (pH 5.1) and extracted with a mixture of hexane and ethyl acetate. The extracts may be methylated to improve chromatographic performance and injected into a GC equipped with an NPD or FID for quantitation or a GC equipped with an MSD for simultaneous quantitation/confirmation. All drugs must be confirmed by GC-MS.

12.2 Specimen Requirements

1 mL of fluid(s) or 1 g of tissue(s) or comparable amounts of fluid or tissue dilutions/homogenates.

12.3 Reagents and Standards

- Butalbital, 1 mg/mL
- Pentobarbital, 1 mg/mL
- Secobarbital, 1 mg/mL
- Phenobarbital, 1 mg/mL
- Amobarbital, 1 mg/mL
- Butabarbital, 1 mg/mL
- Glutethimide, 1 mg/mL
- Thiopental, 1 mg/mL
- Cyclopentobarbital (cyclopal), internal standard
- MethElute™ (TMPAH, trimethylphenylammonium hydroxide), stored at room temperature
- Monosodium phosphate
- Hexane
- Ethyl acetate

12.4 Solutions, Internal Standards, Calibrators and Controls

12.4.1 1.5 M monosodium phosphate buffer: Add 103.4 grams of monosodium phosphate (NaH_2PO_4) to a 500 mL volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

12.4.2 Hexane:ethyl acetate (50:50): Mix 500 mL hexane with 500 mL ethyl acetate. Store at room temperature for up to two years.

12.4.3 Internal Standard: Weigh 20 mg of cyclopal free acid, transfer to a 10 mL volumetric flask and qs to volume with methanol for final concentration of 2 mg/mL. Dilute 100 µL of 2 mg/mL internal standard solution with 900 µL methanol. Add 50 µL of diluted internal standard to each sample. Alternatively, mix internal standard with extraction solvent.

12.4.4 Extraction solvent for option 1 containing internal standard: Aliquot 2 mL of 2 mg/mL cyclopentobarbital (cyclopal) stock solution into a 1000 mL volumetric flask and qs to volume with extraction solvent (hexane:ethyl acetate) to yield 4 mg/L cyclopal in extraction solvent. Store at room temperature for up to two years.

12.4.5 Alternative internal standards (such as phensuximide, hexobarbital, secobarbital and glutethimide) may be utilized provided they are not present in case samples.

12.4.6 Drug stock solutions:

If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free acid, transfer to a 10 mL volumetric flask and qs to volume with methanol. Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free acid, and weigh this amount.

12.4.7 Working Standard Solution A (0.1 mg/mL): Add 1.0 ml of each of the following 1 mg/mL stock solutions to a 10 mL volumetric flask and qs to volume with methanol: butalbital and phenobarbital.

12.4.8 Working Standard Solution B (0.1 mg/mL): Add 1.0 ml of each of the following stock solutions to a 10 mL volumetric flask and qs to volume with methanol: amobarbital, butabarbital, secobarbital, pentobarbital, and glutethimide.

12.4.9 Blood calibrators, standards, and controls preparation:

12.4.9.1 To prepare the following calibration curve, pipet the following volumes of working standard solution A into appropriately labeled 16 x 125 mm screw cap test tubes

- 30 mg/L Calibrator 300 µL of working standard solution A
- 20 mg/L Calibrator 200 µL of working standard solution A
- 10 mg/L Calibrator 100 µL of working standard solution A
- 5 mg/L Calibrator 50 µL of working standard solution A
- 2 mg/L Calibrator 20 µL of working standard solution A
- 1 mg/L Calibrator 10 µL of working standard solution A

- To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood.

- Add 1 mL blank blood to each tube.

12.4.9.2 Standard B contains rarely encountered drugs (amobarbital, butabarbital, secobarbital, pentobarbital, and glutethimide). During routine barbiturate analyses, run at least 1 standard containing working solution B for retention times. If any of the 4 drugs are present, a full calibration curve is required.

12.4.9.2.1 For routine analyses, pipet 100 µL of working Standard Solution B into a 16 x 125 mm labeled screw-cap test tube. Evaporate to dryness under nitrogen. Add 1 mL blank blood for a final concentration of 10 mg/L.

12.4.9.2.2 If a full calibration curve is required, pipet the following volumes of working standard solution B into appropriately labeled 16 x 125 mm screw cap test tubes

- 30 mg/L Calibrator 300 µL of working standard solution B
- 20 mg/L Calibrator 200 µL of working standard solution B
- 10 mg/L Calibrator 100 µL of working standard solution B
- 5 mg/L Calibrator 50 µL of working standard solution B
- 2 mg/L Calibrator 20 µL of working standard solution B
- 1 mg/L Calibrator 10 µL of working standard solution B

12.4.9.2.3 To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood.

12.4.9.2.4 Add 1 mL blank blood to each tube.

12.4.9.3 Standards A and B may be combined to quantitate all 9 drugs simultaneously.

12.4.9.4 This procedure may be used to quantitate other acidic and neutral drugs such as primidone, metaxalone, and thiopental. Prepare calibrators in similar manner as the barbiturates as described above.

12.4.9.5 Controls

12.4.9.5.1 Negative control: Blood bank blood (or comparable) determined not to contain analytes of interest.

12.4.9.5.2 Positive control: Commercial whole blood control and/or an in-house control containing each analyte of interest from a different lot number or manufacturer than standards, or prepared by a chemist different than the one performing the extraction.

12.5 Apparatus

12.5.1 Agilent GC-MS, ChemStation software, compatible computer & printerAgilent GC with NPD or FID, ChemStation software, compatible computer & printer

12.5.2 Test tubes, round bottom, screw cap tubes, borosilicate glass with Teflon caps

12.5.3 Test tubes, glass, conical bottom

12.5.4 Centrifuge capable of 2,000 – 3,000 rpm

12.5.5 Vortex mixer

12.5.6 Evaporator/concentrator

12.5.7 GC autosampler vials and inserts

12.5.8 Test tube rotator

12.5.9 GC-NPD parameters. Instrument conditions may be changed to permit improved performance.

12.5.9.1 Oven program

- Equilibration time: 0.50 minutes
- Initial temp: 110°C
- Initial time: 1.0 minute
- Ramp: 15°C/min
- Final Temp: 280°C
- Final Time: 4 minutes
- Run Time: 15 minutes

12.5.9.2 Inlet

- Mode: Splitless
- Temperature: 250°C
- Constant pressure: 16 psi
- Purge flow: 49.6 mL/min
- Total flow: 52.9 mL/min
- Injection volume: 1.0 µL

12.5.9.3 Detector

- Temperature: 290°C
- Hydrogen flow: 3.0. mL/min
- Air flow: 60 mL/min
- Mode: Constant column + makeup flow

- Combined flow: 20.0 mL/min
- Injection volume: 1.0 µL
- Makeup flow: On

12.5.9.4 Column: HP 1 or 5, 30 m x 0.25 mm x 0.25 µm.

12.5.10 GC-FID parameters. Conditions may be changed to permit improved performance.

12.5.10.1 Oven program

- Equilibration time: 0.50 minutes
- Initial temp: 110°C
- Initial time: 1.0 minutes
- Ramp: 15°C/min
- Final Temp: 260°C
- Final Time: 1.5 minutes
- Run Time: 15 minutes

12.5.10.2 Inlet

- Mode: Splitless
- Temperature: 250°C
- Constant pressure: 25 psi
- Purge flow: 1.9 mL/min
- Total flow: 6.1 mL/min
- Injection volume: 1.0 µL

12.5.10.3 Detector

12.5.10.4 Column: HP-5, 30 m x 0.25 mm x 0.25 µm.

12.5.11 GC-MS parameters. Instrument conditions may be changed to permit improved performance.

12.5.11.1 Acquisition Mode: Scan (50 – 550 amu) or SIM

12.5.11.2 SIM ions:

butalbital	196, 195, 181
butabarbital	169, 184, 211
amobarbital	169, 184, 225
pentobarbital	169, 184, 225
secobarbital	196, 181, 237
phenobarbital	232, 117, 146
iminostilbene	193, 192, 165
cyclopal	221, 196
glutethimide	203, 132, 117

12.5.11.3 Column: HP 5MS, 30 m x 0.25 mm x 0.25 µm

12.5.11.4 Detector Temperature: 280°C

12.5.11.5 Instrument conditions may be changed to permit improved performance.

12.5.11.5.1 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 110°C
- Initial time: 1 minutes

- Ramp: 15°C/min
- Final Temp: 280°C
- Final Time: 4 minutes
- Run Time: 15 minutes

12.5.11.5.2 Inlet

- Mode: Splitless
- Temperature: 270°C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

12.6 Procedure

12.6.1 Label clean screw cap tubes accordingly, negative, calibrators, control(s) and case sample IDs.

12.6.2 Prepare calibrators and controls.

12.6.3 Pipet 1 mL of each case sample into appropriately labeled tubes.

12.6.4 Add 1 mL 1.5 M sodium phosphate buffer (pH 5.1) to each tube.

12.6.5 Add 3 mL extraction solvent (hexane:ethyl acetate) and internal standard to each tube. The extraction solvent may be premixed with internal standard.

12.6.6 Cap and rotate tubes for 30 minutes.

12.6.7 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Transfer organic (upper) layer to clean conical bottom tubes. Discard lower layers.

12.6.8 Evaporate samples to dryness under nitrogen at 50-55°C.

12.6.9 Add 100 µL MethElute™ to each tube.

12.6.10 Transfer a small aliquot to appropriately labeled GC vials and inject 1-2 µl on GC-NPD, GC-FID or GC-MSD.

12.6.11 Save remainder of reconstituted samples for confirmation by GC-MSD (if not already confirmed).

12.7 Calculation

Calculate the concentrations by interpolation of a linear plot of the response curve based on peak height (or area) ratios versus calibrator concentration.

12.8 Quality Control and Reporting

12.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte.

12.8.2 The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

12.8.3 See Toxicology Quality Guidelines

12.9 References

12.9.1 Stewart, Duke and Willcox. Rapid micromethod for the gas chromatographic determination of methylated barbiturates in biological samples. *Anal Letters* 2: 449-456, 1969.

12.9.2 Brochmann-Hanssen and Oke. Gas chromatography of barbiturates, phenolic alkaloids and xanthine bases: flash-heater methylation by means of trimethylanilinium hydroxide. *J Pharm Sci* 58: 370-371, 1969.

12.9.3 Kananen, Osiewicz and Sunshine. Barbiturate analysis—a current assessment. *J Chrom Sci* 10: 283-287, 1972.

12.9.4 Dwight Flammia, in-house development.

13 CHLORDIAZEPOXIDE QUANTITATION AND CONFIRMATION BY LCMSMS

13.1 Summary

Chlordiazepoxide is extracted from biological samples by adding sodium carbonate buffer and extracting with 1-chlorobutane. An aliquot of the extract is quantitated and confirmed by LCMSMS.

13.2 Specimen Requirements

1 mL blood, fluid or tissue homogenate.

13.3 Reagents and Standards

13.3.1 Drug targets and internal standards

Target	Internal Standard
Chlordiazepoxide	Diazepam-D_5

13.3.2 Sodium carbonate, certified ACS powder

13.3.3 1-chlorobutane, HPLC grade

13.3.4 Acetonitrile, Fisher Optima grade or higher grade

13.3.5 Type I or LCMS grade water

13.3.6 Formic acid, eluent additive for LCMS

13.4 Solutions, Internal Standard, Calibrators and Controls

13.4.1 0.2 M Sodium carbonate: weigh out 10.6 g sodium carbonate, transfer to a 500 mL volumetric flask and qs to volume with dH_2O. Store at room temperature for up to 2 years.

13.4.2 Mobile Phase A (H_2O with 0.1% formic acid): add 1 mL of formic acid to 1 L of Type I or LCMS grade H_2O. Store at room temperature for up to one month.

13.4.3 Mobile Phase B (Acetonitrile with 0.1% formic acid): add 1 mL of formic acid to 1 L of acetonitrile. Store at room temperature for up to one month.

13.4.4 Preparation of calibrators.

13.4.4.1 Working standard solution (0.1 mg/mL): Pipette 1.0 mL of the 1 mg/mL stock solution into a 10 mL volumetric flask and qs to volume with methanol. Working standard solution shall be prepared fresh daily.

13.4.4.2 Working standard solution (0.01 mg/mL): pipette 1.0 mL of the 0.1 mg/mL working standard solution into a 10 mL volumetric flask and qs to volume with methanol. Working standard solution shall be prepared fresh daily.

13.4.4.3 Working internal standard solution (0.1 mg/mL): Pipette 1.0 mL of the 1.0 mg/mL stock solution of deuterated standard into a 10 mL volumetric flask and qs to volume with methanol.

13.4.4.4 To prepare calibration curve, pipette the following volumes of the 0.1 mg/mL or 0.01 mg/mL working standard solution into appropriately labeled screw cap test tubes. To eliminate a

solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 1 mL blank blood to obtain the final concentrations listed below.

Amount of 0.1 mg/mL stock solution (µL)	Amount of 0.01 mg/mL stock solution (µL)	Final concentration of chlordiazepoxide (mg/L)
100		10.0
80		8.0
60		6.0
50		5.0
40		4.0
20	200	2.0
10	100	1.0
-	50	0.5

13.4.5 Controls

13.4.5.1 Chlordiazepoxide Control. Controls may be from an external source or prepared in-house using drugs from different manufacturers or lot numbers.

Note: Due to the quadratic nature of chlordiazepoxide, at least three controls, at low, medium and high concentrations, must be run across the concentration range in every batch. If the high calibrator is 10 mg/L, a high control must be run within 8-10 mg/L.

13.4.5.2 Negative control. Blood bank blood or equivalent determined not to contain chlordiazepoxide.

13.5 Apparatus

13.5.1 Test tubes, round bottom, borosilicate glass with Teflon caps

13.5.2 Test tubes, conical bottom

13.5.3 Centrifuge capable of 2000-3000 rpm

13.5.4 Evaporator/concentrator

13.5.5 Vortex mixer

13.5.6 GC autosampler vials with inserts

13.5.7 Typical LCMSMS parameters. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

13.5.7.1 LC Parameters

- Column: Poroshell 120 EC-C18, 2.1x75 mm, 2.7 µm particle size
- Column Thermostat: 30°C
- Mobile Phase A: H_2O with 0.1% formic acid
- Mobile Phase B: Acetonitrile with 0.1% formic acid
- Initial Flow Rate: 0.50 mL/min
- Injection vol.: 1 µL with 20 second needle wash
- Stop Time: 5 min
- Post Run Time: 1.5 min

- Gradient:

Time (minutes)	%A	%B
0.00	80	20
4.00	5	95
4.50	5	95
5.00	80	20

13.5.7.2 Typical MS-MS parameters. The following instrument conditions may be modified to adjust or improve sensitivity.

- MSD Parameters:

Ionization: ESI
Polarity: Positive
Gas temp: 350°C
Drying Gas: 10.0 L/min
Nebulizer press: 50 psi
Capillary: 4000 V

- MRM Parameters

Time Segment Number	Time Segment (minutes)	Diverter Valve
TS1	0.0-0.8	Waste
TS2	0.8-2.0	Chlordiazepoxide
TS3	2.0-3.5	Diazepam-D_5
TS4	3.5-5.0	Waste

- Transition Ions

Target/Internal Standard	Precursor Ion	Product Ions
Diazepam-D_5	290	198, 154
Chlordiazepoxide	300	227, 89

13.6 Procedure

13.6.1 Label clean screw cap tubes appropriately with calibrators, controls and case sample IDs.

13.6.2 Prepare calibrators and controls.

13.6.3 Add 1.0 mL case specimens to the appropriately labeled tubes.

13.6.4 Add 30 µL of the 0.1 mg/mL internal standard working solution to each tube and vortex.

13.6.5 Add 1 mL sodium carbonate and 6 mL 1-chlorobutane to each tube.

13.6.6 Cap and rotate tubes for 30 minutes.

13.6.7 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Transfer organic (upper) layer to appropriately labeled tubes.

13.6.8 Evaporate samples to dryness at approximately 50°C under nitrogen.

13.6.9 Reconstitute samples in 1.0 mL methanol. Transfer to GC autosampler vials with inserts for LCMSMS analysis.

13.7 Quality Control and Reporting

13.7.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte.

13.7.2 The upper limit of quantitation (ULOQ) for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

13.7.3 The calibration model for chlordiazepoxide is weighted (1/x) quadratic. Samples with a concentration greater than 8 mg/L for chlordiazepoxide must be repeated if the high positive control is outside of acceptable limits.

13.7.4 When a target concentration is above the ULOQ, 1.0 mL of case sample shall be diluted with no more than 19.0 mL of blank blood for a total dilution volume of 20.0 mL. Alternatively, 0.05 mL of case sample may be used for a dilution of 1:20. If a dilution greater than 1:10 is required, a positive displacement pipette shall be used for the delivery of case sample. If less than 0.05 mL of sample is used for analysis, only qualitative results may be reported.

13.7.5 Extracted samples are stable for twenty-four hours.

13.7.6 See Toxicology Quality Guidelines.

13.8 References

13.8.1 Wagner, R.L., McLean, L. Virginia Department of Forensic Science In-house method development chlordiazepoxide quantitation and confirmation by LCMSMS. **2016**.

13.8.2 J.S. Hudson, J.W. Hutchings, P. Friel, benzodiazepine in-house development. **2011**.

13.8.3 Melo, P; Bastos, M.L.; Teixeira, H.M. Benzodiazepine stability in postmortem samples stored at different temperatures. *Journal of Analytical Toxicology*. **2012**, 36, 52-60.

13.8.4 Simonsen, K.W.; Hermansson, S.; Steentoft, A.; Linnet, K. A validated method for simultaneous screening and quantification of twenty-three benzodiazepines and metabolites plus zopiclone and zaleplon in whole blood by liquid-liquid extraction and ultra-performance liquid chromatography-tandem mass spectrometry. *Journal of Analytical Toxicology*. **2010**, 34, 332-341.

13.8.5 Wong, A. An evaluation of HPLC for the screening and quantitation of benzodiazepines and acetaminophen in post mortem blood. *Journal of Analytical Toxicology*. **1983**, 7, 33-36.

13.8.6 Levine, B.; Blanke, R.V.; Valentour, J.C. Postmortem stability of benzodiazepines in blood and tissues. *Journal of Forensic Sciences*. **1983**, 1, 102-115.

14 CARISOPRODOL AND MEPROBAMATE QUANTITATION AND CONFIRMATION BY GC AND GC-MS

14.1 Summary

Biological samples are buffered with phosphate buffer (pH 7) and extracted with a mixture of hexane and ethyl acetate. The extract is washed with hexane and reconstituted with toluene/hexane/isoamyl alcohol. An aliquot is injected into a GC equipped with an FID detector for quantitation of carisoprodol and meprobamate. The aliquot can be subsequently injected into a GC-MS for confirmation, if necessary.

14.2 Specimen Requirements

200 µl biological fluid or comparable amount of tissue dilutions/homogenates.

14.3 Reagents and Standards

- Carisoprodol
- Meprobamate
- Cyclopal (cyclopentobarbital) or methaqualone used as internal standards
- Disodium phosphate (Na_2HPO_4)
- Monosodium phosphate (NaH_2PO_4)
- Hexane
- Isoamyl alcohol
- Methanol
- Toluene
- Ethyl acetate
- Acetonitrile
- N-chlorobutane
- MethElute™ (TMPAH, trimethylphenylammonium hydroxide), stored at room temperature

14.4 Solutions, Internal Standard, Calibrators and Controls

14.4.1 0.1 M disodium phosphate: Weigh 14.19 g of disodium phosphate, transfer to a 1 L volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

14.4.2 0.1 M monosodium phosphate: Weigh 13.79 g monosodium phosphate and transfer to a 1 L volumetric flask. qs to volume with dH_2O. Store at room temperature for up to two years.

14.4.3 0.1 M sodium phosphate buffer (pH 7.0): Mix 500 mL 0.1 M disodium phosphate with approximately 250 mL 0.1 M monosodium phosphate. Adjust pH to 7.0 ± 0.1 with 0.1 M monosodium phosphate (lowers pH) or 0.1 M disodium phosphate (raises pH). Store at room temperature for up to two years.

14.4.4 Toluene:Hexane:Isoamyl Alcohol (THIA) (78:20:2, v:v:v): Mix 78 mL toluene, 20 mL hexane and 2 mL isoamyl alcohol. Store at room temperature for up to two years.

14.4.5 Hexane/ethyl acetate (50:50, v:v) extraction solvent: Mix 50 mL hexane with 50 mL ethyl acetate. Store at room temperature for up to two years.

14.4.6 Methanol/ dH_2O (50:50, v:v): Mix 50 mL methanol with 50 mL dH_2O. Store at room temperature for up to two years.

14.4.7 Drug stock solutions:

14.4.7.1 If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and qs to volume with methanol.

14.4.7.2 Working Standard (0.1 mg/mL): Pipette 100 µL of 1.0 mg/mL stock carisoprodol solution, 100 µL of 1.0 mg/mL stock meprobamate solution into a 1 mL volumetric flask and qs to volume with methanol.Alternate volumes may be used to prepare the working standard, provided the final working standard concentrations remains 0.1 mg/mL.

14.4.7.3 Internal standard solution (methylated cyclopal (cyclopentobarbital)): To 1 mL of 2 mg/mL cyclopal (in methanol), add 1 mL MethElute™. Cap and heat at 60°C for 2 hours. Let sit at room temperature overnight. Evaporate under nitrogen. Reconstitute with 10 mL methanol/ dH_2O (50:50, v:v).

14.4.7.4 Methaqualone working stock internal standard. Dilute stock 1 mg/mL 1:10 to prepare 0.1 mg/mL working stock. Methaqualone does not need to be derivatized with TMSH.

14.4.8 Blood calibrators, standards, and controls preparation:

14.4.8.1 To prepare the calibration curve, pipet the following volumes of 1 mg/mL carisoprodol and meprobamate stock solutions into appropriately labeled 13 x 100 mm screw cap test tubes (Note: 100 mg/L Calibrator may overload column, this calibrator may be removed).
Note: For options with both solutions listed, choose either the stock solutions or the working standard solution.

Calibrator Concentration (mg/L)	Volume of 1.0 mg/mL of each stock solution (µL)	Volume of 0.1 mg/mL working standard solution (µL)
100	300	
50	150	
20	60	
10 (see note)	30	300
5 (see note)	15	150
2		60

14.4.8.2 To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood.

14.4.8.3 Add 3 mL blank blood to each tube. Store at 2-8° C for up to one year. Allow standards to equilibrate for 24 hours prior to use.

14.4.8.4 Controls

14.4.8.4.1 Negative control. Blood bank blood (or comparable) determined not to contain carisoprodol or meprobamate

14.4.8.4.2 Positive control: Commercial whole blood control and/or an in-house control containing each analyte of interest from a different lot number or manufacturer than standards, or prepared by a chemist different than the one performing the extraction.

14.5 Apparatus

14.5.1 Agilent GC-MS, ChemStation software (for confirmation, if necessary)

14.5.2 Agilent GC equipped with Flame Ionization Detector, ChemStation software, compatible computer & printer

14.5.3 Test tubes round bottom, screw cap tubes, borosilicate glass with Teflon caps

14.5.4 Test tubes, glass centrifuge, conical bottom

14.5.5 Centrifuge capable of 2,000 – 3,000 rpm

14.5.6 Vortex mixer

14.5.7 Evaporator/concentrator

14.5.8 GC autosampler vials and inserts

14.5.9 Test tube rotator

14.5.10 GC-FID parameters. Conditions may be changed to permit improved performance.

14.5.10.1 Oven program.

- Equilibration time: 0.50 minutes
- Initial temp: 110°C
- Initial time: 1.0 minutes
- Ramp: 20°C/min
- Final Temp: 260°C
- Final Time: 1.5 minutes
- Run Time: 15 minutes

14.5.10.2 Inlet.

- Mode: Splitless
- Temperature: 250°C
- Constant pressure: 25 psi
- Purge flow: 1.9 mL/min
- Total flow: 6.1 mL/min
- Injection volume: 1.0 µL

14.5.10.3 Detector.

- Temperature: 290°C
- Hydrogen flow: 50 mL/min
- Air flow: 450 mL/min
- Mode: Constant makeup flow
- Makeup flow: 45 mL/min

14.5.10.4 Column: HP-5, 30 m x 0.25 mm x 0.25 µm.

14.5.11 GC-MS parameters. Conditions may be changed to permit improved performance.

14.5.11.1 Acquisition Mode: Scan (50 – 550 amu)

14.5.11.2 Column: HP 5MS, 30 m x 0.25 mm x 0.25 µm

14.5.11.3 Detector Temperature: 280°C

14.5.11.4 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 110°C
- Initial time: 1 minutes
- Ramp: 10°C/min
- Final Temp: 290°C

- Final Time: 9 minutes
- Run Time: 28 minutes

14.5.11.4.1 Inlet

- Mode: Splitless
- Temperature: 270°C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

14.6 Procedure

14.6.1 Label clean screw cap tubes accordingly, negative, calibrators, control(s) and case sample IDs.

14.6.2 Prepare calibrators and controls

14.6.3 Pipet 200 µL of each calibrator, control, negative and case samples into appropriately labeled tubes.

14.6.4 Add 30 µL methylated cyclopal or 30 µL methaqualone internal standard to each tube.

14.6.5 Add 0.5 mL sodium phosphate buffer (pH 7) to each tube.

14.6.6 Add 3 mL extract solvent (hexane/ethyl acetate) to each tube.

14.6.7 Cap and rotate tubes for 15 minutes.

14.6.8 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Transfer organic upper layer to clean conical bottom centrifuge tubes. Discard lower layers.

14.6.9 Evaporate samples to dryness under nitrogen at 50-60°C.

14.6.10 Reconstitute samples with 0.2 mL acetonitrile. Vortex briefly.

14.6.11 Add 1 mL hexane to each tube. Vortex each sample for 30 seconds.

14.6.12 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

14.6.13 Aspirate (and discard) upper (hexane) layer.

14.6.14 Evaporate lower (acetonitrile) layer under nitrogen at 50-60°C.

14.6.15 Reconstitute samples with 75 µL of toluene/hexane/isoamyl alcohol solvent or n-chlorobutane and vortex briefly. The reconstitution volume may be modified as necessary to increase/decrease instrument response (e.g., use 150 µL solvent to reconstitute highest calibrator to prevent detector saturation).

14.6.16 Transfer samples to appropriately labeled GC vials and inject 1-2 µl on GC-FID.

14.6.17 Save remainder of reconstituted samples for confirmation by GC-MSD (if not already confirmed).

14.7 Calculation

Calculate the concentrations by interpolation of a linear plot of the response curve based on peak height (or area) ratios versus calibrator concentration.

14.8 Quality Control and Reporting

14.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte. The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

14.8.2 See Toxicology Quality Guidelines

14.9 References

14.9.1 In-house development, T England.

15 GHB QUANTITATION AND CONFIRMATION BY GC-MS

15.1 Summary

GHB is extracted from biological samples by acidifying samples with sulfuric acid followed by extraction with ethyl acetate. The extracts are derivatized with BSTFA and analyzed by GC-MS using selected ion monitoring.

15.2 Specimen Requirements

Approximately 200 µL of blood, biological fluids or tissue homogenates.

15.3 Reagents and Standards

- Gamma hydroxybutyrate (GHB), 1 mg/mL
- 1, 4 butanediol, 1 mg/mL
- GHB-d_6, 1 mg/mL
- Ethyl acetate
- Acetonitrile
- BSTFA, stored at 2-8°C
- Sulfuric acid
- Methanol

15.4 Solutions, Internal Standard, Calibrators and Controls

15.4.1 0.1 N sulfuric acid: Pipet 300 µL concentrated sulfuric acid into a 100 mL volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

15.4.2 GHB working solution (0.2 mg/mL): Pipet 1 mL GHB standard (1 mg/mL) into a 5 mL volumetric flask and qs to volume with methanol.

15.4.3 GHB-d_6 internal standard working solution (0.2 mg/mL): Pipet 1 mL GHB-d_6 internal standard (1 mg/mL) into a 5 mL volumetric flask and qs to volume with methanol.

15.4.4 Blood calibrator and control preparation:

15.4.4.1 Add the following volumes of GHB working solution to appropriately labeled 16 x 125 mm screw cap tubes for the following final concentrations of GHB:

400 mg/L GHB: 80 µL GHB 1 mg/mL standard

200 mg/L GHB: 40 µL GHB 1 mg/mL standard

100 mg/L GHB: 20 µL GHB 1 mg/mL standard

40 mg/L GHB: 40 µL GHB 0.2 mg/mL working solution

20 mg/L GHB: 20 µL GHB 0.2 mg/mL working solution

10 mg/L GHB: 10 µL GHB 0.2 mg/mL working solution

15.4.4.1.1 To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 200 µL blank blood to each calibrator.

15.4.4.1.2 Negative control. Blood bank blood (or comparable) determined not to contain GHB.

15.4.4.1.3 Positive control. Control may be from an external source or prepared in-house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

15.4.4.1.4 1,4 butanediol control. Add 100 µL of 1 mg/mL 1,4 butanediol standard to a calibrator or blank blood for retention time.

15.5 Apparatus

15.5.1 Agilent GC-MS, ChemStation software, compatible computer and printer

15.5.2 Test tubes, round bottom, screw cap tubes, borosilicate glass with Teflon caps

15.5.3 Test tubes, glass centrifuge, conical bottom

15.5.4 Centrifuge capable of 2,000 – 3,000 rpm

15.5.5 Vortex mixer

15.5.6 Heating block

15.5.7 Evaporator/concentrator

15.5.8 GC autosampler vials and inserts

15.5.9 GC-MS Parameters. Instrument conditions may be changed to permit improved performance.

15.5.9.1 Acquisition Mode: SIM (quantitation ions)

GHB: 233, 117, 204
GHB d_6: 239

15.5.9.2 Column: HP5 MS, 30 m x 0.25 mm x 0.25 µm

15.5.9.3 Detector Temperature: 280°C

15.5.9.4 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 80°C
- Initial time: 2 minutes
- Ramp: 17°C/min
- Final Temp: 280°C
- Final Time: 5 minutes
- Run Time 15 minutes

15.5.9.4.1 Inlet

- Mode: Split, ratio 10:1
- Temperature: 250°C
- Injection volume: 1.0 µL
- Purge Time: ON at 2.0 minute

15.6 Procedure

15.6.1 Label clean screw cap tubes accordingly, negative, calibrators, controls and case sample IDs.

15.6.2 Prepare calibrators and controls.

15.6.3 Pipet 200 µL of each case sample into appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case. One will serve as a screen and the second as a confirmation.

15.6.4 Add 200 µL GHB-d_6 internal standard working solution (0.2 mg/mL) to each tube.

15.6.5 Add 250 µL cold 0.1N sulfuric acid to each tube.

15.6.6 Add 6 mL ethyl acetate to each tube.

15.6.7 Vortex each sample for 30 seconds.

15.6.8 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

15.6.9 Transfer upper organic layer (ethyl acetate) to labeled conical bottom tubes.

15.6.10 Evaporate samples under nitrogen at 55°C.

15.6.11 Add 60 µL acetonitrile to each sample.

15.6.12 Add 30 µL BSTFA to each sample. Mix briefly.

15.6.13 Heat samples at 70°C for 15 minutes.

15.6.14 Transfer samples to GC autosampler vials.

15.6.15 Inject samples on GC-MS.

15.7 Calculation

Calculate the concentrations by interpolation of a linear plot of the response curve based on ratios (using target ions listed under GC-MS conditions) versus calibrator concentration.

15.8 Quality Control and Reporting

15.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte. The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

15.8.2 If GHB is positive (> 20 mg/L), GHB must be confirmed in a second extract using the same procedure.

15.8.3 If the same specimen is analyzed in duplicate (for screening and confirmation) and both results are quantitative, the results should be averaged prior to reporting.

15.8.4 For postmortem interpretation, GHB analysis should be conducted on blood and an additional fluid such as urine or vitreous to help discriminate between GHB consumption and postmortem production.

15.8.5 See Toxicology Quality Guidelines

15.9 References

15.9.1 A solid phase method for GHB in urine without conversion to GBL, United Chemical Technologies.

15.9.2 F. Couper and B. Logan. Determination of GHB in biological specimens by GC-MS. J Anal Tox 24:1-7, 2000.

15.9.3 J Pearson and M Blackburn. Analysis of GHB in whole blood and urine by GC-MS. J Anal Tox 25(5): 366, 2001.

15.9.4 Randall Edwards, in-house development.

16 VALPROIC ACID QUANTITATION AND CONFIRMATION BY GC-MS

16.1 Summary

Biological samples are slightly acidified with monosodium phosphate buffer (pH 5.1) and extracted with toluene/hexane/isoamyl alcohol (THIA). An aliquot is injected into a GC-MS for quantitation and confirmation.

16.2 Specimen Requirements

1 mL biological fluid or comparable amount of tissue dilutions/homogenates.

16.3 Reagents and Standards

- Phensuximide
- Valproic acid
- Monosodium phosphate (NaH_2PO_4)•H_20
- Toluene
- Hexane
- Isoamyl Alcohol
- Methanol

16.4 Solutions, Internal Standard, Calibrators and Controls

16.4.1 1.5 M monosodium phosphate: Weigh 103.4 g monosodium phosphate (NaH_2PO_4•H_20) and transfer to a 500 mL volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

16.4.2 Toluene:Hexane:Isoamyl Alcohol (THIA) (78:20:2, v:v:v): Mix 78 mL toluene, 20 mL hexane and 2 mL isoamyl alcohol. Store at room temperature for up to 2 years.

16.4.3 1 mg/mL valproic acid stock solution. Weigh 10 mg valproic acid, transfer to a 10 mL volumetric flask and qs to volume with methanol.

16.4.4 1 mg/mL phensuximide internal standard solution. Weigh 10 mg phensuximide, transfer to a 10 mL volumetric flask and qs to volume with methanol.

16.4.5 Calibrators and controls

16.4.5.1 To prepare the calibration curve, pipet the following volumes of 1 mg/mL valproic acid into appropriately labeled 13 x 100 mm screw cap test tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 1 mL blank blood to achieve final concentration.

- 400 mg/L Calibrator 400 µl valproic acid (1 mg/mL)
- 200 mg/L Calibrator 200 µl valproic acid (1 mg/mL)
- 100 mg/L Calibrator 100 µL valproic acid (1 mg/mL)
- 50 mg/L Calibrator 50 µL valproic acid (1 mg/mL)
- 25 mg/L Calibrator 25 µL valproic acid (1 mg/mL)

16.4.5.2 Negative control. Blood bank blood (or comparable) determined not to contain valproic acid or phensuximide.

16.4.5.3 Positive control. In-house control containing valproic acid spiked at concentration similar to case specimens.

16.5 Apparatus

16.5.1 Agilent GC-MS, ChemStation software

16.5.2 Test tubes, glass centrifuge, conical bottom

16.5.3 Centrifuge capable of 2000 – 3000 rpm

16.5.4 Vortex mixer

16.5.5 GC autosampler vials and inserts

16.5.6 GC-MS parameters. Conditions may be changed to permit improved performance.

16.5.6.1 Acquisition Mode: SIM

16.5.6.2 SIM ions: valproic acid 73, 102, 115
phensuximide 104, 189, 78

16.5.6.3 Column: HP 5, 30 m x 0.25 mm x 0.25 µm

16.5.6.4 Detector Temperature: 280°C

16.5.6.5 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 50°C
- Initial time: 1 minutes
- Ramp: 15°C/min
- Final Temp: 280°C
- Final Time: 1 minute
- Run Time: 15 minutes

17.5.6.5.1 Inlet

- Mode: Splitless
- Temperature: 260°C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

16.6 Procedure

16.6.1 Label clean screw cap tubes accordingly, negative and positive control and case sample IDs.

16.6.2 Prepare calibrators and controls.

16.6.3 Pipet 1 mL of each case sample into appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case. One will serve as a screen and the second as a confirmation.

16.6.4 Add 10 µL of 1 mg/mL phensuximide internal standard to each tube for a final concentration of 10 mg/L.

16.6.5 Add 1 mL sodium monophosphate buffer (pH 5.5) to each tube.

16.6.6 Add 1 mL THIA to each tube.

16.6.7 Vortex briefly.

16.6.8 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Remove a portion of upper organic layer to appropriately labeled GC vials. Note: samples may be diluted to prevent saturation of the detector.

16.6.9 Inject 1 µl of each sample onto GC-MS.

16.7 Calculation

Drug concentrations are calculated by linear regression analysis using ChemStation software based on peak height (or area) ratios versus calibrator concentration.

16.8 Quality Control and Reporting

16.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte. The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

16.8.2 If the same specimen is analyzed in duplicate (for screening and confirmation) and both results are quantitative, the results should be averaged prior to reporting.

16.8.3 See Toxicology Quality Guidelines

16.9 References

16.9.1 I. Sunshine. Methodology for Analytical Toxicology. CRC Press, 1982.

16.9.2 Carol O'Neal, Amy Jango, Lucy Sale and Dwight Flammia, in-house development.

17 FENTANYL QUANTITATION AND CONFIRMATION BY GC-MS

17.1 Summary

Fentanyl, acetyl fentanyl, butyryl fentanyl, and desproprionyl fentanyl are extracted from biological fluids and tissues using solid phase or liquid-liquid extraction followed by instrumental analysis by GC-MS using selected ion monitoring. (Note: not all targets may be run every time but by customer request)

17.2 Specimen Requirements

2 mL whole blood, urine, bile, gastric contents, other fluids or tissue homogenates.

17.3 Reagents and Standards

- Ammonium hydroxide
- Glacial Acetic Acid
- Potassium Hydroxide
- Potassium Phosphate
- Ethyl Acetate
- Methanol
- Acetonitrile
- Dichloromethane
- Isopropyl alcohol
- Hexane
- Toluene
- Isoamyl alcohol
- Potassium or sodium phosphate buffer solution concentrate (1 M, pH 6.0, e.g., Fisher)
- Sodium phosphate, monobasic ($NaH_2PO_4 \cdot H_20$)
- Sodium phosphate, dibasic (Na_2HPO_4)
- Sodium tetraborate decahydrate
- Sodium hydroxide
- Sodium hydrogen carbonate
- Potassium carbonate
- Sulfuric acid
- Fentanyl, 100 µg/mL
- Fentanyl d-5, 100 µg/mL
- Acetyl Fentanyl
- Butyryl Fentanyl
- Furanyl Fentanyl
- Desproprionyl Fentanyl
- cis-3-methylfentanyl
- trans-3-methylfentanyl
- para-fluorobutyrylfentanyl
- para-fluoroisobutyrylfentanyl

17.4 Solutions, Internal Standards, Calibrators and Controls

17.4.1 Solutions for Varian SPE Extraction (Option 1)

17.4.1.1 1 M Acetic Acid. Add 5.75 mL of glacial Acetic Acid to a 100 mL volumetric flask half filled with dH_2O and qs to volume with dH_2O. Store at room temperature for up to two years.

17.4.1.2 5.0 M Potassium Hydroxide. Weigh 28 g of potassium hydroxide into a 100 mL beaker containing approximately 70 mL dH_2O. After the potassium hydroxide has dissolved, transfer

to 100 mL volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

17.4.1.3 0.1 M Phosphate Buffer, pH 6.0. Weigh 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5.0 M potassium hydroxide while stirring and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Store at room temperature for up to two years.

17.4.1.4 2% Ammonium Hydroxide in Ethyl Acetate. Pipette 2 mL of concentrated ammonium hydroxide into a 100 mL graduated cylinder filled with 98 mL of ethyl acetate. Cap graduated cylinder with a glass stopper, and mix well. Vent cylinder occasionally. PREPARE SOLUTION FRESH DAILY!

17.4.2 Solutions for UCT CleanScreen® SPE Extraction (Option 2)

17.4.2.1 When using UCT CleanScreen® SPE Extraction columns, either sodium or potassium phosphate buffer may be used. However, the same buffer (sodium or phosphate) must be used throughout the duration of the procedure.

17.4.2.1.1 0.1 M Potassium Phosphate Buffer, pH 6.0. Weigh 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5 M potassium hydroxide while stirring and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Store at room temperature for up to two years.

OR

17.4.2.1.2 0.1 M Sodium Phosphate Buffer, pH 6.0. Weigh 1.70g Na_2HPO_4 and 12.14g $NaH_2PO_4 \cdot H_2O$ and transfer to a 1 L volumetric flask containing approximately 800 mL dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5 M sodium hydroxide and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Store at room temperature for up to two years.

17.4.2.2 1.0 M Acetic Acid. Add 5.75 mL of glacial Acetic Acid to a 100 mL volumetric flask half filled with dH_2O and qs to volume with dH_2O. Store at room temperature for up to two years.

17.4.2.3 Dichloromethane/isopropanol/ammonium hydroxide (78:20:2). Mix 78 mL dichloromethane with 20 mL isopropanol. Mix well. In hood, add 2 mL ammonium hydroxide. Mix gently. PREPARE SOLUTION FRESH DAILY!

17.4.3 Solutions for liquid/liquid base extraction (Option 3)

17.4.3.1 Saturated borate buffer solution. Add sodium tetraborate decahydrate to dH_2O until no more dissolves after shaking vigorously. Decant saturated solution into a glass jar equipped with a volumetric dispenser. Store at room temperature for up to two years.

17.4.3.2 Toluene:Hexane:Isoamyl Alcohol (THIA) extraction solvent (78:20:2), v:v:v: Mix toluene (780 mL), hexane (200 mL), and isoamyl alcohol (20 mL). Store at room temperature for up to two years.

17.4.3.3 Sodium Hydrogen Carbonate/Potassium Carbonate (dry 3:2 w/w) Mix 300 g $NaHCO_3$ with 200 g K_2CO_3. Store at room temperature for up to two years.

17.4.3.4 0.5 N Sulfuric Acid: Add 13.8 mL concentrated sulfuric acid to a 1 L volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

17.4.4 Internal Standard

Working internal standard solution (1 µg/mL fentanyl d-5): Pipet 100 µL of the 100 µg/mL fentanyl d-5 stock solution into a 10 mL volumetric flask and qs to volume with methanol.

17.4.5 Calibrators

17.4.5.1 Working standard solutions:

17.4.5.1.1 (1 µg/mL fentanyl): Pipet 100 µL of the 100 µg/mL fentanyl standard stock solution into a 10 mL volumetric flask and qs to volume with methanol.

17.4.5.1.2 (0.1 µg/mL fentanyl): Pipet 1 mL of the 1 µg/mL fentanyl working solution into a 10 mL volumetric flask and qs to volume with methanol.

17.4.5.2 To prepare the calibration curve, pipet the following volumes of working standard solution into 16 x 125 mm tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 2 mL blank blood to each tube to obtain the final concentrations listed below:

Final Concentration of fentanyl (mg/L)	µL of fentanyl working solution (1 µg/mL)	(0.1 µg/mL)
0.001		20
0.002		40
0.004	8	80
0.010	20	200
0.025	50	
0.050	100	
0.100 (optional for tissues)	200	

17.4.6 Controls

17.4.6.1 Working control solution (1 µg/mL fentanyl): Pipet 100 µL of a 100 µg/mL fentanyl stock solution (manufacturer or lot number different than that used for calibrators) into 10 mL volumetric flask and qs to volume with methanol. Store in freezer. Qualitative only target controls can be prepared using this procedure.

17.4.6.2 The following is an example of how to prepare positive controls. Other controls (at different concentrations) may be prepared to monitor the performance of the assay. To prepare in-house controls, pipet the following volumes of control standard solution into 16 x 125 mm tubes. Add 2 mL blank blood to each tube to obtain the final concentrations listed below:

Final Concentration of fentanyl (mg/L)	µL of fentanyl working solution (1 µg/mL)
0.005	10
0.020	40

17.4.6.3 Commercial whole blood control.

17.4.6.4 Negative blood control: Blood bank blood or equivalent previously determined not to contain fentanyl.

17.5 Apparatus

17.5.1 Agilent GC-MS, ChemStation software, compatible computer & printer

17.5.2 Test tubes, round bottom, screw cap tubes, borosilicate glass with Teflon caps

17.5.3 Test tubes, round bottom tubes, borosilicate glass

17.5.4 Test tubes, glass centrifuge, conical bottom

17.5.5 Test tubes, round bottom, screw cap tubes, borosilicate glass

17.5.6 Centrifuge capable of 2,000 – 3,000 rpm

17.5.7 Varian Bond Elute LRC Certify solid phase extraction (SPE) columns or Cleanscreen® Extraction Cartridges (ZSDAU020) from United Chemical Technologies (200 mg columns)

17.5.8 Solid phase extraction manifold

17.5.9 Vortex mixer

17.5.10 Evaporator/concentrator

17.5.11 GC autosampler vials and inserts

17.5.12 Test tube rotator

17.5.13 GC-MS parameters. Instrument conditions may be changed to permit improved performance. Underscored ion is used for quantitation.

17.5.13.1 Acquisition Mode: SIM

Fentanyl:	<u>245</u>, 146, 189
Acetyl Fentanyl	231,146,188 (qualitative only)
Butyryl Fentanyl	259, 146, 189 (qualitative only)
Desproprionyl Fentanyl:	146,189,118 (qualitative only)
trans-3-methylfentanyl:	259, 203, 216 (qualitative only)
cis-3-methylfentanyl:	259, 160, 203 (qualitative only)
Fluorobutyrylfentanyl:	277, 105, 164 (qualitative only)
Fluoroisobutyrylfentanyl:	277, 105, 164 (qualitative only)
Furanyl Fentanyl:	283, 240, 158 (qualitative only)
Fentanyl d-5:	<u>250</u>, 151

17.5.13.2 Column: HP-5MS 30 m x 0.25 mm x 0.25 µm

17.5.13.3 Detector Temperature: 280°C

17.5.13.4 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 140°C
- Initial time: 1 minutes
- Ramp: 20°C /min
- Final Temp: 290°C
- Final Time: 4 minutes
- Run Time: 9 minutes

17.5.13.5 Inlet

- Mode: Pulsed splitless
- Temperature: 270°C
- Injection volume: 2.0 µL
- Purge Time: ON at 1.0 minute

17.6 Procedure

17.6.1 Extraction Option 1, Varian SPE Columns

17.6.1.1 Label screw cap tubes accordingly.

17.6.1.2 Pipet 2 mL of blank blood, calibrators, controls and case sample bloods, fluids or tissue homogenates into appropriately labeled tubes.

17.6.1.3 Pipet 20 µL internal standard working solution into all tubes for a final concentration of 0.010 mg/L fentanyl d-5. Vortex briefly.

17.6.1.4 Add 6 mL of acetonitrile, cap and immediately shake each tube. Put tubes on mechanical rotator for 10 minutes. Note: urine samples do not require acetonitrile precipitation of proteins (go to 18.6.1.8).

17.6.1.5 Centrifuge tubes at approximately 2500 rpm for 15 minutes to achieve separation.

17.6.1.6 Decant acetonitrile supernatant into labeled, disposable, borosilicate glass culture tubes.

17.6.1.7 Evaporate acetonitrile to 1-2 mL in an evaporator/concentrator.

17.6.1.8 Add 2 mL of pH 6.0, 0.1 M potassium phosphate buffer to all tubes and vortex.

17.6.1.9 Solid phase extraction (SPE). Place SPE cartridges in the extraction manifold. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

17.6.1.9.1 Condition columns with 2 mL methanol and aspirate.

17.6.1.9.2 Add 2 mL pH 6.0 phosphate buffer and aspirate.

17.6.1.9.3 Pour specimens into appropriate SPE columns. Aspirate slowly so that the sample takes at least 2 minutes to pass through the column.

17.6.1.9.4 Add 6 mL dH_2O and aspirate < 3 inches of mercury.

17.6.1.9.5 Wash with 3 mL 1 M acetic acid and aspirate.

17.6.1.9.6 Dry the columns at > 10 inches of Hg for approximately 10 minutes.

17.6.1.9.7 Add 6 mL methanol and aspirate under full vacuum/pressure for at least 2 minutes.

17.6.1.9.8 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

17.6.1.9.9 Elute fentanyl by adding 2 mL of ammonium hydroxide:ethyl acetate (2:98) to each column. Collect eluate in conical test tubes by column aspiration or gravity drain.

17.6.1.10 Evaporate eluates to dryness at approximately 50°C under nitrogen.

17.6.1.11 Add 100 µL ethyl acetate to the extracts. Vortex and transfer to autosampler vials for analysis by GC-MS. Note: reconstitution volume may be adjusted to prevent saturation of the detector.

17.6.2 Extraction Option 2, CleanScreen SPE Columns.

17.6.2.1 Label clean screw cap tubes accordingly.

17.6.2.2 Pipet 2 mL of blank blood, calibrators, controls and case sample bloods, fluids or tissue homogenates into appropriately labeled tubes.

17.6.2.3 Pipet 20 µL internal standard working solution into all tubes for a final concentration of 0.010 mg/L fentanyl d-5. Vortex briefly.

17.6.2.4 Add 4 mL deionized water to each tube. Mix, vortex briefly and let stand for 5 minutes.

17.6.2.5 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Transfer supernatant to clean 16 x 125 mm tubes and discard the tube with the remaining pellet.

17.6.2.6 Add 2 mL of pH 6 phosphate buffer, mix and vortex. As necessary adjust the pH to 5.5 to 6.5 with additional 0.1 M phosphate buffer. Note: larger volumes of water or phosphate buffer may be used to further dilute some specimens prior to SPE analysis.

17.6.2.7 Solid phase extraction. Place SPE cartridges in the extraction manifold. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

17.6.2.7.1 Add 3 mL hexane to each column and aspirate.

17.6.2.7.2 Add 3 mL methanol to each column and aspirate.

17.6.2.7.3 Add 3 mL dH_2O and aspirate.

17.6.2.7.4 Add 1 mL of 0.1 M pH 6.0 phosphate buffer and aspirate.

17.6.2.7.5 Without delay, pour specimens into appropriate SPE columns. Elute from cartridges under vacuum at approximately 1-2 mL/ minute flow.

17.6.2.7.6 Add 3 mL dH_2O and aspirate at ≤ 3inches of mercury.

17.6.2.7.7 Repeat the dH_2O wash.

17.6.2.7.8 Wash with 2.0 mL 1.0 M acetic acid and aspirate.

17.6.2.7.9 Add 3 mL methanol and aspirate under full vacuum/pressure for at least 5 minutes

17.6.2.7.10 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

17.6.2.7.11 Elute fentanyl by adding 3 mL of freshly prepared methylene chloride/isopropanol/ammonium hydroxide solution to each column. Collect eluate in conical test tubes by column aspiration or gravity drain.

17.6.2.8 Evaporate eluates to dryness at approximately 50°C under nitrogen.

17.6.2.9 Reconstitute the residue with 100 µL of toluene/hexane/isoamyl alcohol, vortex and transfer to autosampler vials for analysis by GC-MS. Note: reconstitution volume may be adjusted to prevent saturation of the detector.

17.6.3 Extraction Option 3, Basic LLE

17.6.3.1 Label clean screw cap tubes accordingly.

17.6.3.2 Pipet 2 mL of blank blood, calibrators, controls and case sample bloods, fluids or tissue homogenates into appropriately labeled tubes.

17.6.3.3 Pipet 20 µL internal standard working solution into all tubes for a final concentration of 0.010 mg/L fentanyl d-5. Vortex briefly.

17.6.3.4 Add 2 mL of saturated borate buffer to each tube.

17.6.3.5 Add 4 mL of toluene/hexane/isoamyl alcohol extraction solvent to each tube.

17.6.3.6 Rotate tubes for 20 minutes.

17.6.3.7 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

17.6.3.8 Transfer the top (organic) layer to appropriately labeled 13 x 100 mm screw-cap test tubes. Discard lower (aqueous) layer.

17.6.3.9 Add 2 mL of 0.5 N sulfuric acid to tubes. Cap and rotate 20 minutes. Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

17.6.3.10 Aspirate off top (organic) layer and discard.

17.6.3.11 Adjust aqueous layer to a basic pH by slowly adding solid 3:2 $NaHCO_3/K_2CO_3$ buffer until effervescence ceases. Then add approximately 0.3 g excess $NaHCO_3/K_2CO_3$ buffer to saturate the aqueous layer.

17.6.3.12 Add 200 µL of toluene/ hexane/isoamyl alcohol extraction solvent to each tube, cap tubes and vortex for 10-15 seconds. Centrifuge tubes at approximately 2500 rpm for 15 minutes to achieve separation.

17.6.3.13 Transfer approximately 100 µL of top (organic) layer into GC autosampler vials for analysis by GC-MS. Note: reconstitution volume may be adjusted to prevent saturation of the detector.

17.7 Calculation

Quantitation. Prepare a response curve of area (height) of analyte to area (height) of internal standard ratio versus calibrator concentration. Calculate the analyte concentration by interpolation of the linear plot.

17.8 Quality Control and Reporting

17.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte. The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

17.8.2 For tissues containing high concentrations of fentanyl, an additional high calibrator may be used to extend the ULOQ to 0.100 mg/L fentanyl, as necessary.

17.8.3 Due to the extremely low concentrations of fentanyl, it is advantageous to collect GC-MS data in µg/mL or ng/mL and then convert to mg/L for reporting.

17.8.4 See Toxicology Quality Guidelines.

17.9 References

17.9.1 Varian Bond Elute Certify™ Instruction Manual

17.9.2 J Kuhlman, R McCaulley, T Valouch and G Behonick. Fentanyl Use, Misuse and Abuse: A Summary of 23 Postmortem Cases. J. Anal. Toxicol. 2003; Oct (27): 499-504.

17.9.3 W.H. Anderson and D.C. Fuller, "A Simplified Procedure for the Isolation, Characterization, and Identification of Weak Acid and Neutral Drugs from Whole Blood." J. Anal. Toxicol. 1987, Sep/Oct (11): 198-204.

18 QUETIAPINE QUANTITATION AND CONFIRMATION BY GC-MS

18.1 Summary

Quetiapine is extracted from biological fluids and tissues using solid phase or liquid-liquid extraction followed by derivatization with BSTFA and instrumental analysis by GC-MS using selected ion monitoring.

18.2 Specimen Requirements

2 mL whole blood, urine, bile, gastric contents, other fluids or tissue homogenates.

18.3 Reagents and Standards

- Ammonium hydroxide
- Glacial Acetic Acid
- Potassium Hydroxide
- Potassium Phosphate
- Ethyl Acetate
- Methanol
- Acetonitrile
- Dichloromethane
- Isopropyl alcohol
- Hexane
- Toluene
- Isoamyl alcohol
- Potassium or sodium phosphate buffer solution concentrate (1 M, pH 6.0, e.g., Fisher)
- Sodium phosphate, monobasic ($NaH_2PO_4 \bullet H_20$)
- Sodium phosphate, dibasic (Na_2HPO_4)
- Sodium tetraborate decahydrate
- Sodium hydroxide
- Sodium hydrogen carbonate
- Potassium carbonate
- Sulfuric acid
- BSTFA containing 1% TMCS, stored at 2-8°C
- Quetiapine, 1 mg/mL
- Methapyrilene, 1 mg/mL

18.4 Solutions, Internal Standards, Calibrators and Controls

18.4.1 Solutions for Varian SPE Extraction (Option 1)

18.4.1.1 1 M Acetic Acid. Add 5.75 mL of glacial Acetic Acid to a 100 mL volumetric flask half filled with dH_2O and qs to volume with dH_2O. Store at room temperature for up to two years.

18.4.1.2 5.0 M Potassium Hydroxide. Weigh 28 g of potassium hydroxide into a 100 mL beaker containing approximately 70 mL dH_2O. After the potassium hydroxide has dissolved, transfer to 100 mL volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

18.4.1.3 0.1 M Phosphate Buffer, pH 6.0. Weigh 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5.0 M potassium hydroxide while stirring and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Store at room temperature for up to two years.

18.4.1.4 2% Ammonium Hydroxide in Ethyl Acetate. Pipette 2 mL of concentrated ammonium hydroxide into a 100 mL graduated cylinder filled with 98 mL of ethyl acetate. Cap graduated cylinder with a glass stopper, and mix well. Vent cylinder occasionally. PREPARE SOLUTION FRESH DAILY!

18.4.2 Solutions for UCT CleanScreen® SPE Extraction (Option 2)

18.4.2.1 When using UCT CleanScreen® SPE Extraction columns, either sodium or potassium phosphate buffer may be used. However, the same buffer (sodium or phosphate) must be used throughout the duration of the procedure.

18.4.2.1.1 0.1 M Potassium Phosphate Buffer, pH 6.0. Weigh 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5 M potassium hydroxide while stirring and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Store at room temperature for up to two years.

OR

18.4.2.1.2 0.1 M Sodium Phosphate Buffer, pH 6.0. Weigh 1.70g Na_2HPO_4 and 12.14g $NaH_2PO_4 \cdot H_2O$ and transfer to a 1 L volumetric flask containing approximately 800 mL dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5 M sodium hydroxide and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Store at room temperature for up to two years.

18.4.2.2 1.0 M Acetic Acid. Add 5.75 mL of glacial Acetic Acid to a 100 mL volumetric flask half filled with dH_2O and qs to volume with dH_2O. Store at room temperature for up to two years.

18.4.2.3 Dichloromethane/isopropanol/ammonium hydroxide (78:20:2). Mix 78 mL dichloromethane with 20 mL isopropanol. Mix well. In hood, add 2 mL ammonium hydroxide. Mix gently. PREPARE SOLUTION FRESH DAILY!

18.4.3 Solutions for liquid/liquid base extraction (Option 3)

18.4.3.1 Saturated borate buffer solution. Add sodium tetraborate decahydrate to dH_2O until no more dissolves after shaking vigorously. Decant saturated solution into a glass jar equipped with a volumetric dispenser. Store at room temperature for up to two years.

18.4.3.2 Toluene:Hexane:Isoamyl Alcohol (THIA) extraction solvent (78:20:2), v:v:v: Mix toluene (780 mL), hexane (200 mL), and isoamyl alcohol (20 mL). Store at room temperature for up to two years.

18.4.3.3 Sodium Hydrogen Carbonate/Potassium Carbonate (dry 3:2 w/w) Mix 300 g $NaHCO_3$ with 200 g K_2CO_3. Store at room temperature for up to two years.

18.4.3.4 0.5 N Sulfuric Acid: Add 13.8 mL concentrated sulfuric acid to a 1 L volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

18.4.4 Internal Standard

Working internal standard solution (0.01 mg/mL methapyrilene): Pipet 100 µL of the 1 mg/mL methapyrilene stock solution into a 10 mL volumetric flask and qs to volume with methanol.

18.4.5 Calibrators

18.4.5.1 Working standard solution (0.02 mg/mL quetiapine): Pipet 100 µL of the 1 mg/mL quetiapine standard stock solution into a 5 mL volumetric flask and qs to volume with methanol.

18.4.5.2 To prepare the calibration curve, pipet the following volumes of working standard solution into 16 x 125 mm tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 2 mL blank blood to each tube to obtain the final concentrations listed below:

Final Concentration of quetiapine (mg/L)	µL of quetiapine solution (0.02 mg/mL)
0.10	10
0.20	20
0.40	40
1.0	100
2.0	200
5.0	500

18.4.6 Controls

18.4.6.1 Control may be from an external source or prepared in-house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

18.4.6.2 Negative control. Blood bank blood or equivalent determined not to contain quetiapine.

18.5 Apparatus

18.5.1 Agilent GC-MS, ChemStation software, compatible computer & printer

18.5.2 Test tubes, round bottom, screw cap tubes, borosilicate glass with Teflon caps

18.5.3 Test tubes, round bottom tubes, borosilicate glass

18.5.4 Test tubes, glass centrifuge, conical bottom

18.5.5 Test tubes, round bottom, screw cap tubes, borosilicate glass

18.5.6 Centrifuge capable of 2,000 – 3,000 rpm

18.5.7 Varian Bond Elute LRC Certify solid phase extraction (SPE) columns or Cleanscreen® Extraction Cartridges (ZSDAU020) from United Chemical Technologies (200 mg columns)

18.5.8 Solid phase extraction manifold

18.5.9 Vortex mixer

18.5.10 Evaporator/concentrator

18.5.11 GC autosampler vials and inserts

18.5.12 Test tube rotator

18.5.13 GC-MS parameters. Instrument conditions may be changed to permit improved performance. Underscored ion is used for quantitation.

18.5.13.1 Acquisition Mode: SIM

Quetiapine: 210, 239, 322

Methapyrilene: 58, 97

18.5.13.2 Column: HP-5MS 30 m x 0.25 mm x 0.25 µm

18.5.13.3 Detector Temperature: 280°C

18.5.13.4 Column: HP 5MS 30 m x 0.25 mm x 0.25 µm

18.5.13.5 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 140°C
- Initial time: 0 minutes
- Ramp: 30°C/min
- Final Temp: 300°C
- Final Time: 7 minutes
- Run Time: 12.5 minutes

18.5.13.6 Inlet

- Mode: Pulsed splitless
- Temperature: 270°C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

18.6 Procedure

18.6.1 Extraction Option 1, Varian SPE Columns

18.6.1.1 Label screw cap tubes accordingly.

18.6.1.2 Pipet 2 mL of blank blood, calibrators, controls and case sample bloods, fluids or tissue homogenates into appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case. One will serve as a screen and the second as a confirmation (unless quetiapine has already been confirmed using the base screen procedure).

18.6.1.3 Pipet 100 µL internal standard working solution into all tubes for a final concentration of 0.5 mg/L methapyrilene. Vortex briefly.

18.6.1.4 Add 6 mL of acetonitrile, cap and immediately shake each tube. Put tubes on mechanical rotator for 10 minutes. Note: urine samples do not require acetonitrile precipitation of proteins (go to step 19.6.1.8).

18.6.1.5 Centrifuge tubes at approximately 2500 rpm for 15 minutes to achieve separation.

18.6.1.6 Decant acetonitrile supernatant into labeled, disposable, borosilicate glass culture tubes.

18.6.1.7 Evaporate acetonitrile to 1-2 mL in an evaporator/concentrator.

18.6.1.8 Add 2 mL of pH 6.0, 0.1 M potassium phosphate buffer to all tubes and vortex. Note: larger volumes of water or phosphate buffer may be used to further dilute some specimens prior to SPE analysis.

18.6.1.9 Solid phase extraction (SPE). Place SPE cartridges in the extraction manifold. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

18.6.1.9.1 Condition columns with 2 mL methanol and aspirate.

18.6.1.9.2 Add 2 mL pH 6.0 phosphate buffer and aspirate.

18.6.1.9.3 Pour specimens into appropriate SPE columns. Aspirate slowly so that the sample takes at least 2 minutes to pass through the column.

18.6.1.9.4 Add 6 mL dH_2O and aspirate < 3 inches of mercury.

18.6.1.9.5 Wash with 3 mL 1 M acetic acid and aspirate.

18.6.1.9.6 Dry the columns at > 10 inches of Hg for approximately 10 minutes.

18.6.1.9.7 Add 6 mL methanol and aspirate under full vacuum/pressure for at least 2 minutes.

18.6.1.9.8 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

18.6.1.9.9 Elute quetiapine by adding 2 mL of ammonium hydroxide:ethyl acetate(2:98) to each column. Collect eluate in conical test tubes by column aspiration or gravity drain.

18.6.1.10 Evaporate eluates to dryness at approximately 50-60°C under nitrogen.

18.6.1.11 Add 50 µL BSTFA and 50 µL ethyl acetate to the extracts. Heat at 90°C for 30 minutes.

18.6.1.12 Transfer to autosampler vials for analysis by GC-MS.

18.6.2 Extraction Option 2, CleanScreen SPE Columns.

18.6.2.1 Label clean screw cap tubes accordingly.

18.6.2.2 Pipet 2 mL of blank blood, calibrators, controls and case sample bloods, fluids or tissue homogenates into appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case. One will serve as a screen and the second as a confirmation (unless quetiapine has already been confirmed using the base screen procedure).

18.6.2.3 Pipet 100 µL internal standard working solution into all tubes for a final concentration of 0.5 mg/L methapyrilene. Vortex briefly.

18.6.2.4 Add 4 mL deionized water to each tube. Mix, vortex briefly and let stand for 5 minutes.

18.6.2.5 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Transfer supernatant to clean tubes and discard the tube with the remaining pellet.

18.6.2.6 Add 2 mL of pH 6 phosphate buffer, mix and vortex. As necessary adjust the pH to 5.5 to 6.5 with additional 0.1 M phosphate buffer. Note: larger volumes of water or phosphate buffer may be used to further dilute some specimens prior to SPE analysis.

18.6.2.7 Solid phase extraction. Place SPE cartridges in the extraction manifold. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

18.6.2.7.1 Add 3 mL hexane to each column and aspirate.

18.6.2.7.2 Add 3 mL methanol to each column and aspirate.

18.6.2.7.3 Add 3 mL dH_2O and aspirate.

18.6.2.7.4 Add 1 mL of 0.1 M pH 6.0 phosphate buffer and aspirate.

18.6.2.7.5 Without delay, pour specimens into appropriate SPE columns. Elute from cartridges under vacuum at approximately 1-2 mL/ minute flow.

18.6.2.7.6 Add 3 mL dH_2O and aspirate at ≤ 3inches of mercury.

18.6.2.7.7 Repeat the dH_2O wash.

18.6.2.7.8 Wash with 2.0 mL 1.0 M acetic acid and aspirate.

18.6.2.7.9 Add 3 mL methanol and aspirate under full vacuum/pressure for at least 5 minutes.

18.6.2.7.10 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

18.6.2.7.11 Elute quetiapine by adding 3 mL of freshly prepared methylene chloride/isopropanol/ammonium hydroxide solution to each column. Collect eluate in conical test tubes by column aspiration or gravity drain.

18.6.2.8 Evaporate eluates to dryness at approximately 50-60°C under nitrogen.

18.6.2.9 Add 50 µL BSTFA and 50 µL ethyl acetate to the extracts. Heat at 90°C for 30 minutes.

18.6.2.10 Transfer to autosampler vials for analysis by GC-MS.

18.6.3 Extraction Option 3, Basic LLE

18.6.3.1 Label clean screw cap tubes accordingly.

18.6.3.2 Pipet 2 mL of blank blood, calibrators, controls and case sample bloods, fluids or tissue homogenates into appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case. One will serve as a screen and the second as a confirmation (unless quetiapine has already been confirmed using the base screen procedure).

18.6.3.3 Pipet 100 µL internal standard working solution into all tubes for a final concentration of 0.5 mg/L methapyrilene. Vortex briefly.

18.6.3.4 Add 2 mL of saturated borate buffer to each tube.

18.6.3.5 Add 4 mL of toluene/hexane/isoamyl alcohol extraction solvent to each tube.

18.6.3.6 Rotate tubes for 20 minutes.

18.6.3.7 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

18.6.3.8 Transfer the top (organic) layer to appropriately labeled screw-cap test tubes. Discard lower (aqueous) layer.

18.6.3.9 Add 2 mL of 0.5 N sulfuric acid to tubes. Cap and rotate 20 minutes. Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation.

18.6.3.10 Aspirate off top (organic) layer and discard.

18.6.3.11 Adjust aqueous layer to a basic pH by slowly adding solid 3:2 $NaHCO_3/K_2CO_3$ buffer until effervescence ceases. Then add approximately 0.3 g excess $NaHCO_3/K_2CO_3$ buffer to saturate the aqueous layer.

18.6.3.12 Add 200 µL of toluene/hexane/isoamyl alcohol extraction solvent to each tube, cap tubes and vortex for 10-15 seconds. Centrifuge tubes at approximately 2500 rpm for 15 minutes to achieve separation.

18.6.3.13 Transfer approximately 200 µL of top (organic) layer into conical bottom vials. Evaporate to dryness at approximately 55°C under nitrogen.

18.6.3.14 Add 50 µL BSTFA and 50 µL ethyl acetate to the extracts. Heat at 90°C for 30 minutes.

18.6.3.15 Transfer to autosampler vials for analysis by GC-MS.

18.7 Calculation

Quantitation. Prepare a response curve of area (height) of analyte to area (height) of internal standard ratio versus calibrator concentration. Calculate the analyte concentration by interpolation of the linear plot.

18.8 Quality Control and Reporting

18.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte. The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

18.8.2 If the same specimen is analyzed in duplicate (for screening and confirmation) and both results are quantitative, the results should be averaged prior to reporting.

18.8.3 See Toxicology Quality Guidelines.

18.9 References

18.9.1 Varian Bond Elute Certify™ Instruction Manual

18.9.2 D Anderson and K Fritz. Quetiapine (Seroquel) Concentrations in Seven Postmortem Cases. J. Anal. Toxicol. 2000; May/June (24): 300-304.

18.9.3 D Flammia, T Valouch and S Venuti. Tissue Distribution of Quetiapine in 20 Cases in Virginia. J. Anal. Toxicol. 2006, May (30): 287-292.

19 HYDROXYZINE QUANTITATION AND CONFIRMATION BY GC-MS

19.1 Summary

Hydroxyzine is extracted from biological fluids or tissues using solid phase extraction (SPE), derivatized with BSTFA and analyzed by GC-MS in SIM mode.

19.2 Specimen Requirements

- 2 mL whole blood, urine, bile, gastric contents, other fluids or tissue homogenates

19.3 Reagents and Standards

- Ammonium hydroxide
- Glacial Acetic Acid
- Potassium Hydroxide
- Potassium Phosphate
- Ethyl Acetate
- Methanol
- Acetonitrile
- Hexane
- Dichloromethane
- Isopropyl alcohol
- Potassium or sodium phosphate buffer solution concentrate (1 M, pH 6.0, e.g., Fisher)
- Sodium phosphate, monobasic ($NaH_2PO_4 \cdot H_20$)
- Sodium phosphate, dibasic (Na_2HPO_4)
- Anhydrous sodium sulfate
- BSTFA with 1% TMCS, stored at 2-8°C
- Hydroxyzine, 1 mg/mL
- Methapyrilene, 1 mg/mL

19.4 Solutions, Internal Standards, Calibrators and Controls

19.4.1 Solutions for Varian SPE Extraction

19.4.1.1 1 M Acetic Acid. Add 5.75 mL of glacial Acetic Acid to a 100 mL volumetric flask half filled with dH_2O and qs to volume with dH_2O. Store at room temperature for up to two years.

19.4.1.2 5.0 M Potassium Hydroxide. Weigh 28 g of potassium hydroxide into a 100 mL beaker containing approximately 70 mL dH_2O. After the potassium hydroxide has dissolved, transfer to 100 mL volumetric flask and qs to volume with dH_2O. Store at room temperature for up to two years.

19.4.1.3 0.1 M Phosphate Buffer, pH 6.0. Weigh 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5.0 M potassium hydroxide while stirring and qs to volume with dH_2O. Store at room temperature for up to two years.

19.4.1.3.1 Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Dilute one volume potassium phosphate buffer solution concentrate with nine volumes of dH_2O. Store at room temperature for up to two years.

19.4.1.4 2% Ammonium Hydroxide in Ethyl Acetate. Pipette 2 mL of concentrated ammonium hydroxide into a 100 mL graduated cylinder filled with 98 mL of ethyl acetate. Cap graduated

cylinder with a glass stopper, and mix well. Vent cylinder occasionally. PREPARE SOLUTION FRESH DAILY!

19.4.2 Solutions for UCT CleanScreen® SPE Extraction

19.4.2.1 When using UCT CleanScreen® SPE Extraction columns, either sodium or potassium phosphate buffer may be used. However, the same buffer (sodium or phosphate) must be used throughout the duration of the procedure.

19.4.2.1.1 0.1 M Potassium Phosphate Buffer, pH 6.0. Weigh 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5 M potassium hydroxide while stirring and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Dilute one volume potassium phosphate buffer solution concentrate with nine volumes of dH_2O. Store at room temperature for up to two years.

OR

19.4.2.1.2 0.1 M Sodium Phosphate Buffer, pH 6.0. Weigh 1.70g Na_2HPO_4 and 12.14g $NaH_2PO_4 \cdot H_2O$ and transfer to a 1 L volumetric flask containing approximately 800 mL dH_2O. Adjust the pH of the above solution to 6.0 by the addition of 5 M sodium hydroxide and qs to volume with dH_2O. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Dilute one volume sodium phosphate buffer solution concentrate with nine volumes of dH_2O. Store at room temperature for up to two years.

19.4.2.2 1.0 M Acetic Acid. Add 5.75 mL of glacial Acetic Acid to a 100 mL volumetric flask half filled with dH_2O and qs to volume with dH_2O. Store at room temperature for up to two years.

19.4.2.3 Ethyl acetate/Hexane, 50:50 v/v. Mix 500 mL ethyl acetate with 500 mL hexane. Store at room temperature for up to two years.

19.4.2.4 Dichloromethane/isopropanol/ammonium hydroxide (78:20:2). Mix 78 mL dichloromethane with 20 mL isopropanol. Mix well. In hood, add 2 mL ammonium hydroxide. Mix gently. PREPARE SOLUTION FRESH DAILY!

19.4.3 Reconstitution solvent (50:50 hexane:ethyl acetate): Mix 50 mL hexane with 50 mL ethyl acetate. Add enough anhydrous sodium sulfate until no more dissolves after shaking vigorously. Store at room temperature for up to two years.

19.4.4 Internal Standard

Working internal standard solution (0.01 mg/mL methapyrilene or other suitable IS): Pipet 100 µL of the 1 mg/mL stock solution of methapyrilene into a 10 mL volumetric flask and qs to volume with methanol.

19.4.5 Preparation of calibrators.

19.4.5.1 Working hydroxyzine standard solution (0.02 mg/mL). Pipet 100 µL of 1 mg/mL hydroxyzine standard into a 5 mL volumetric flask and qs to volume with methanol.

19.4.5.2 To prepare the calibration curve, pipet the following volumes of the 0.02 mg/mL working hydroxyzine standard solution into appropriately labeled 16 x 125 mm screw cap test tubes.

To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 2 mL blank blood to obtain the final concentrations listed below.

Amount of 0.02 mg/mL hydroxyzine solution (µL)	Final concentration of hydroxyzine (mg/L)
400	4.0
300	3.0
200	2.0
100	1.0
40	0.4
20	0.2
10	0.1

19.4.6 Hydroxyzine Control. Control may be from an external source or prepared in-house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

19.4.7 Negative control. Blood bank blood or equivalent determined not to contain hydroxyzine.

19.5 Apparatus

19.5.1 Agilent GC-MS, ChemStation software, compatible computer & printer

19.5.2 Test tubes, round bottom, screw cap tubes, borosilicate glass with Teflon caps

19.5.3 Test tubes, round bottom tubes, borosilicate glass

19.5.4 Test tubes, glass centrifuge, conical bottom

19.5.5 Test tubes, round bottom, screw cap tubes, borosilicate glass

19.5.6 Centrifuge capable of 2,000 – 3,000 rpm

19.5.7 Varian Bond Elute LRC Certify solid phase extraction (SPE) columns or Cleanscreen® Extraction Cartridges (ZSDAU020) from United Chemical Technologies (200 mg columns)

19.5.8 Solid phase extraction manifold

19.5.9 Vortex mixer

19.5.10 Evaporator/concentrator

19.5.11 Test tube rotator

19.5.12 GC autosampler vials and inserts

19.5.13 GC-MS parameters. Instrument conditions may be changed to permit improved performance.

19.5.13.1 Acquisition Mode: SIM

Hydroxyzine: 201, 165, 299

Methapyrilene: 58, 97

19.5.13.2 Column: HP1 or 5MS, 30 m x 0.25 mm x 0.25 µm

19.5.13.3 Detector Temperature: 280°C

19.5.13.4 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp: 110°C
- Initial time: 1 minute
- Ramp: 10°C /min
- Final Temp: 290°C
- Final Time: 9 minutes
- Run Time: 28 minutes

19.5.13.5 Inlet

- Mode: Splitless
- Temperature: 270°C
- Injection volume: 1.0 µL
- Purge Time: ON at 1.0 minute

19.6 Procedure

19.6.1 Extraction Option 1, Varian SPE Columns

19.6.1.1 Label screw cap tubes accordingly.

19.6.1.2 Pipet 2 mL of corresponding negative and positive control bloods and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case. One will serve as a screen and the second as a confirmation.

19.6.1.3 Add 100 µL working internal standard solution to all tubes for a final concentration of 0.5 mg/L. Vortex briefly.

19.6.1.4 Add 6 mL of acetonitrile, cap and immediately shake each tube. Put tubes on mechanical rotator for 10 minutes. Note: urine samples do not require acetonitrile precipitation of proteins (start at step 19.6.1.8).

19.6.1.5 Centrifuge tubes at approximately 2500 rpm for 15 minutes to achieve separation.

19.6.1.6 Decant acetonitrile supernatant into labeled, disposable, borosilicate glass culture tubes.

19.6.1.7 Evaporate acetonitrile to 1-2 mL in an evaporator/concentrator.

19.6.1.8 Add 2 mL of pH 6.0, 0.1 M potassium phosphate buffer to all tubes and vortex. Note: larger volumes of water or phosphate buffer may be used to further dilute some specimens prior to SPE analysis.

19.6.1.9 Solid phase extraction (SPE). Place SPE cartridges in the extraction manifold. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

19.6.1.9.1 Condition columns with 2 mL methanol and aspirate.

19.6.1.9.2 Add 2 mL pH 6.0 phosphate buffer and aspirate.

19.6.1.9.3 Pour specimens into appropriate SPE columns. Aspirate slowly so that the sample takes at least 2 minutes to pass through the column.

19.6.1.9.4 Add 1 mL of 1 M acetic acid to each column and aspirate. Dry columns under full vacuum/pressure for at least 5 minutes.

19.6.1.9.5 Add 6 mL methanol and aspirate under full vacuum/pressure for at least 2 minutes

19.6.1.9.6 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

19.6.1.9.7 Elute hydroxyzine by adding 2 mL of ammonium hydroxide:ethyl acetate(2:98) to each column. Collect eluate in conical test tubes by column aspiration or gravity drain.

19.6.1.10 Evaporate eluates to dryness under nitrogen at 50-60°C.

19.6.1.11 Add 50 µL BSFTA containing 1%TMCS. Vortex briefly and heat at 60°C for 30 minutes.

19.6.1.12 Evaporate derivatives to dryness under nitrogen at 50-60°C. Let tubes cool to room temperature.

19.6.1.13 Reconstitute samples with 150 µL reconstitution solvent (50:50 hexane:ethyl acetate) and vortex briefly.

19.6.1.14 Transfer to GC autosampler vials for analysis by GC-MS.

19.6.2 Extraction Option 2, CleanScreen SPE Columns.

19.6.2.1 Label clean screw cap tubes accordingly.

19.6.2.2 Pipet 2 mL of corresponding negative and positive control bloods and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case. One will serve as a screen and the second as a confirmation.

19.6.2.3 Add 100 µL working internal standard solution to all tubes for a final concentration of 0.5 mg/L. Vortex briefly.

19.6.2.4 Add 6.0 mL deionized water to each tube. Mix, vortex briefly and let stand for 5 minutes.

19.6.2.5 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Transfer supernatant to clean tubes and discard the tube with the remaining pellet.

19.6.2.6 Add 2.0 mL of pH 6 phosphate buffer, mix and vortex. As necessary adjust the pH to 5.5 to 6.5 with additional 0.1 M phosphate buffer. Note: larger volumes of water or phosphate buffer may be used to further dilute some specimens prior to SPE analysis.

19.6.2.7 Solid phase extraction. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

19.6.2.7.1 Add 3 mL hexane to each column and aspirate on vacuum manifold

19.6.2.7.2 Add 3 mL methanol to each column and aspirate on vacuum manifold.

19.6.2.7.3 Add 3 mL dH_2O and aspirate.

19.6.2.7.4 Add 1 mL of 0.1 M pH 6.0 phosphate buffer and aspirate

19.6.2.7.5 Without delay, pour specimens into appropriate SPE columns. Elute from cartridges under vacuum at approximately 1-2 mL/ minute flow.

19.6.2.7.6 Add 3 mL dH_2O and aspirate at ≤ 3inches of mercury.

19.6.2.7.7 Repeat the dH_2O wash a second time.

19.6.2.7.8 Wash with 2.0 mL 1.0 M acetic acid and aspirate.

19.6.2.7.9 Add 3 mL methanol, aspirate under full vacuum/pressure for at least 2 minutes

19.6.2.7.10 Add 2 mL hexane to each column. Dry columns at ≥ 10 inches of mercury for five minutes.

19.6.2.7.11 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

19.6.2.7.12 Elute hydroxyzine by adding 3 mL of freshly prepared methylene chloride/isopropanol/ammonium hydroxide solution to each column. Collect eluate in conical test tubes by column aspiration or gravity drain.

19.6.2.7.13 Elute at 1 - 2 mL/minute (no vacuum) and collect eluates.

19.6.2.8 Evaporate eluates to dryness under nitrogen at 50-60°C.

19.6.2.9 Add 50 µL BSTFA and 50 µL ethyl acetate to the extracts. Heat at 60°C for 30 minutes.

19.6.2.10 Transfer to GC autosampler vials for analysis by GC-MS.

19.7 Calculation

Quantitation. Prepare a response curve of area (height) of analyte to area (height) of internal standard ratio versus calibrator concentration. Calculate the analyte concentration by interpolation of the linear plot.

19.8 Quality Control and Reporting

19.8.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte. The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

19.8.2 If the same specimen is analyzed in duplicate (for screening and confirmation) and both results are quantitative, the results should be averaged prior to reporting.

19.8.3 See Toxicology Quality Guidelines SOP for quality control and reporting.

19.9 References

19.9.1 Varian Bond Elute Certify™ Instruction Manual

19.9.2 T. Soriano, C. Jurado, M. Menendez and M. Repetto, “Improved Solid-Phase Extraction Method for Systematic Toxicological Analysis in Biological Fluids.” J. Anal. Toxicol. 2001; March (25): 137-143.

19.9.3 W.H. Anderson and D.C. Fuller, “A Simplified Procedure for the Isolation, Characterization, and Identification of Weak Acid and Neutral Drugs from Whole Blood.” J. Anal. Toxicol. 1987, Sep/Oct (11): 198-204.

20 VOLATILE SCREEN AND CONFIRMATION BY HEADSPACE GC AND GC-MS

20.1 Summary

An aliquot of specimen is diluted semi-automatically with an internal standard (IS) solution (n-propanol) into a glass vial, sealed, and placed in a heated automatic sampler. The concentration of volatiles in a dilute aqueous biological sample is directly proportional to its concentration in the gas phase or headspace. A portion of the resultant headspace vapor above the liquid is automatically injected into a gas chromatograph (GC) equipped with a flame ionization detector (FID). Volatiles are identified by retention time and, whenever possible, confirmed by headspace GC-MS.

20.2 Specimen Requirements

Approximately 2 mL of biological fluid(s) or 2 gm tissue. Alternative specimens include canisters/containers of huffing agent or headspace vials containing various tissues (e.g., lung tissue) or air samples (e.g., tracheal air).

20.3 Reagents and Standards

- Reference standards of volatiles or gases
- N-propanol

20.4 Solutions, Internal Standard, Calibrators and Controls

20.4.1 0.10% stock solution: To prepare a mixture of volatiles of interest, use density to calculate the volumes of each volatile necessary to prepare a 0.10% stock solution. For example, to create a 0.10% stock solution of toluene, ethyl acetate and chloroform, pipet the following volumes into a 100 mL volumetric flask and qs to volume with dH_2O:

Volatile	Density	Volume
Toluene	0.86	116 µL
Ethyl Acetate	0.90	110 µL
Chloroform	1.47	68 µL

20.4.2 Preparation of 0.004% standard solution: Pipet 400 µL of the 0.10% stock solution into a 10 mL volumetric flask and qs to volume with dH_2O. This 0.004% standard is used as the limit of detection and the response of the reported analyte in the case specimen must be greater than that of the analyte in the 0.004% standard.

20.4.3 For analytes that are in the gas phase at room temperature (e.g., 1,1 difluroethane), the following are options for the preparation of a qualitative standard:

20.4.3.1 Capture of primary standard in a headspace vial.

20.4.3.1.1 In a fume hood, flush a 20 mL headspace vial with the analyte (usually in the form of a pressurized cylinder or can) for several seconds.

20.4.3.1.2 While continuing to flush the vial, cap vial with septa and hold down until crimped with aluminum cap.

20.4.3.1.3 Generally, the stock primary standard is of such high concentration that it must be diluted in order to avoid overloading the GC column and/or detector. Serial dilutions of the primary standard may be made into nitrogen purged headspace vials using gastight syringes to transfer aliquots of the headspace from vial to vial.

20.4.3.1.4 The appropriate dilutions for an analyte will have to be determined via trial and error. The analyte peak should not be so large as to cause a shift in retention time of the internal standard.

20.4.3.1.5 The final dilution of the standard should be into a vial containing internal standard so that the relative retention times of the analytes may be determined and used to identify the unknowns in case specimens.

20.4.3.2 Capture of a semi-quantitative primary standard in a methanolic solution.

20.4.3.2.1 Fill a 2 mL volumetric flask to the mark with methanol.

20.4.3.2.2 Weigh flask and tare balance.

20.4.3.2.3 Slowly bubble analyte gas into flask.

20.4.3.2.4 Weigh flask to determine approximate concentration (mg gas/2 mL methanol).

20.4.3.2.5 Dilute standard to approximately 0.004% w/v for the limit of detection standard.

20.4.4 0.03% (v/v) n-propanol internal standard solution. Pipet 300 µL n-propanol into a 1 L volumetric flask and qs to volume with dH_2O. Store at room temperature for up to one year.

20.4.5 Negative blood control: Blood bank blood or equivalent previously determined not to contain volatiles.

20.5 Apparatus

20.5.1 Agilent headspace GC equipped with flame ionization detector, Chemstation software, compatible computer and printer

20.5.2 Agilent GC-MS, Chemstation software, compatible computer and printer

20.5.3 Glass 20 mL (23 x 75 mm) headspace vials with Teflon septa and aluminum seals

20.5.4 Vial seal crimper

20.5.5 Hamilton Microlab Diluter or equivalent

20.5.6 Gastight Hamilton syringes

20.5.7 Headspace GC parameters: The following conditions are recommended starting parameters. Modify the parameters as necessary to achieve optimum separation and detection of analytes. Print out the instrument method and optimum conditions and store with each batch analysis.

20.5.7.1 Note: For approximate retention times of volatiles, see Restek Application Note #59548 on GC Analysis of Commonly Abused Inhalants in Blood Using Rtx BAC1 and Rtx BAC2 Columns. Column. Restek Rtx®-BAC 1 or BAC 2 capillary column.

20.5.7.2 Instrument parameters.

- Column Restek BAC1 or BAC2 or Agilent DB-ALC1 and Agilent DB-ALC2 capillary columns, or Agilent DB-BAC1 UI and DB-BAC2 UI capillary columns
- Oven 40ºC
- Ramp 1 5°C/min to 60°C
- Ramp 2 5°C/min to 80°C, 1 min hold
- Injector 200ºC
- Detector (FID) 250°C

- Hydrogen flow 30 mL/min
- Air flow 400 mL/min
- Make-up flow 20 ml/min
- Make-up gas Helium
- Inlet Split
- Split ratio 1:1
- Split flow 14.2 mL/min
- Total flow 30.6 mL/min
- Pressure 8.9 psi constant pressure mode

20.5.8 Headspace Parameters. The following conditions are recommended starting parameters. Modify the parameters as necessary to achieve optimum operation. Print out the optimum conditions and save them with each batch analysis.

- Incubation Temp 70ºC
- Sample Valve 85ºC
- Transfer Line 95ºC
- GC Cycle 10.0 min
- Sample Equilibration 3.0 min
- Vial Pressurization 0.91 min
- Loop Fill 0.20 min (or 500 µL volume)
- Loop Equilibration 0.05 min
- Sample Inject 1.00 min
- Oven Stabilization 1.0 min
- Agitation Low
- Extractions 1
- Puncture Mode Single

20.5.9 Headspace GC-MS parameters. The following conditions are recommended starting parameters. Modify the parameters as necessary to achieve optimum operation. Print out the optimum conditions and save them with each batch analysis.

- Injection Manual
- Split Mode 10:1 split
- Split flow 12 mL/min
- Injector Temp 200ºC
- Pressure 9.1 psi
- Total flow 15.9 mL/min
- Flow 1.2 ml/min (40 cm/sec)
- Column HP-5MS, 30 m x 0.25 mm ID x 0.25 µm film
- He Flow 1.2 ml/min (40 cm/sec)
- Oven 40°C initial, 0.3 minute hold
- Ramp 10°C/min to 100°C
- Equilibration time 0.50 min
- Scan mass range 45-100 amu
- EM voltage 200
- Threshold 150

20.6 Procedure

20.6.1 Headspace GC-FID Analysis

20.6.1.1 Prepare 0.004% volatile standard or qualitative standard.

20.6.1.2 Pipet 0.2 mL of the volatile standard or qualitative standard, negative control and case specimens into appropriately labeled clean headspace vials. Add 450 µL 0.03% n-propanol to each headspace vial.

20.6.1.2.1 Note: If sample is already in headspace vial (tracheal air or lung), add 450 µL 0.03% n-propanol to the headspace vial using a needle and syringe through the septa/seal. If the submitted sample is to be used for both HS-FID and GCMS (see below), add 1 mL of 0.03% n-propanol to the headspace vial using a needle and syringe through the septa/seal.

20.6.1.2.2 Note: If analyzing weighed tissue specimens, place approximately 0.5 gm weighed tissue in the headspace vial and add 450 µL 0.03% n-propanol to each headspace vial.

20.6.1.3 Stopper each headspace vial with a Teflon seal, as needed. Vortex or manually shake each vial for several seconds and place in the sample rack.

20.6.1.4 Seal all headspace vials by crimping the aluminum rings over the Teflon seals.

20.6.1.5 Load headspace vials in the headspace auto sampler.

20.6.2 Headspace GC-MS Confirmation

20.6.2.1 Prepare 0.01% volatile standard or qualitative standard.

20.6.2.2 Pipet 1 mL of the volatile standard or qualitative standard, negative control and case specimens into appropriately labeled clean headspace vials. Add 1 mL 0.03% n-propanol to each headspace vial.

20.6.2.2.1 Note: If sample is already in headspace vial (tracheal air or lung), add 1 mL 0.03% n-propanol to the headspace vial using a needle and syringe through the septa/seal.

20.6.2.2.2 Note: If analyzing weighed tissue specimens, place approximately 2 gm weighed tissue in the headspace vial and add 1 mL 0.03% n-propanol to each headspace vial.

20.6.2.3 Stopper each headspace vial with a Teflon seal, as needed. Vortex or manually shake each vial for several seconds and place in the sample rack.

20.6.2.4 Seal all headspace vials by crimping the aluminum rings over the Teflon seals.

20.6.2.5 Heat each headspace vial at 80°C for 10 minutes in oven.

20.6.2.6 Use disposable 1 mL gas tight syringe to withdraw 1 mL headspace from each vial and manually inject directly into GC-MS. Note: start MSD 3 seconds prior to injection.

20.6.2.7 Compare specimen unknown mass spectra to known volatile mass spectra.

20.7 Quality Control and Reporting

20.7.1 Volatiles are identified on headspace GC-FID based on relative retention times compared to the volatile standard. Identification is performed by instrument software. Relative retention times should be within ± 2% of the relative retention time obtained from the volatile standard.

20.7.2 Volatiles must be confirmed by headspace GC-MS by comparison of the retention time and mass spectra of the unknown sample to that the retention and mass spectra of a known volatile standard.

20.7.3 The case sample analyte peak height must be more than 5 times greater than the peak height of the negative control.

20.7.4 Volatiles are reported as “present” only.

20.7.5 Exceptions to these guidelines must be justified with supporting analytical documentation, authorized by the Program Manager and documented in the case file with an MFR.

20.8 References

20.8.1 Restek Application Note #59548. GC Analysis of Commonly Abused Inhalants in Blood Using Rtx BAC1 and Rtx BAC2 Columns.

20.8.2 L. C. Nickolls, "A Modified Cavett Method for the Determination of Alcohol in Body Fluids," Nov. 1960, Analyst, Vol. 85, pp 840-942.

20.8.3 B. Kolb, "Head Space Analysis by Means of the Automated Gas Chromatograph F-40 Mulitfract", Bodenseewerk Perkin-Elmer and Co., Technical Manual #15E.

20.8.4 K. M. Dubowski, "Manual for Analysis of Ethanol in Biological Liquids," Department of Transportation Report No. DOT TSC NHTSA-76-4, Jan 1977.

20.8.5 G. Machata, "Determination of Alcohol in Blood by Gas Chromatographic Head Space Analysis," Clin Chem. Newsletter, 4(1972), 29.

20.8.6 B.L. Levine, Principles of Forensic Toxicology, American Association for Clinical Chemistry, Inc., 1999, p. 180.

20.8.7 Randall Edwards, in-house development.

21 HEAVY METALS BY REINSCH TEST

21.1 Summary

Rapid presumptive tests are simple colorimetric tests that may be performed directly on blood, urine, gastric contents or liver with little or no previous sample preparation. When run with negative and positive controls, these tests are sensitive enough to detect overdoses; however since these tests are presumptive only, confirmation should be performed on positive findings.

21.2 Heavy Metal Screen (Reinsch Test)

21.2.1 Principle: certain heavy metals can be quickly and easily identified when ingested in acute toxic doses using the classical Reinsch Test. The Reinsch test identifies arsenic, antimony, bismuth and mercury. The test is based on the ability of metallic copper, in the presence of strong acid, to reduce selected heavy metals to their elemental form (e.g., arsenic is deposited on the copper as a visible dull black film):

$$3Cu^{0} + 2As^{+3} + HCl \rightarrow 3Cu^{+2} + 2As^{0}$$

21.2.2 Reagents and controls

21.2.2.1 Concentrated hydrochloric acid

21.2.2.2 Copper spiral (#20 gauge) or foil strip. Wind copper around a glass rod or pencil. Clean the copper by dunking in concentrated nitric acid for a few seconds, then immediately immerse in water. The copper should be bright and shiny.

21.2.2.3 Arsenic reference solution, 1 mg/mL. Dissolve 0.132 g of arsenic trioxide in 1.0 mL of 10 N sodium hydroxide and add 50 mL dH_2O. Neutralize the solution with concentrated HCl, then qs to 100 mL with dH_2O.

21.2.3 Procedure (urine, gastric contents or liver are preferred specimens)

21.2.3.1 Place clean copper spirals into separate 100 mL beakers or 125 mL Erlenmeyer flasks labeled for negative and positive controls and unknown(s).

21.2.3.2 Place 20 mL urine, approximately 10-15 g minced tissue in 20 mL dH_2O, or an aliquot of gastric contents dissolved in 20 mL dH_2O into a labeled beaker. Place 20 mL negative control urine in two separate beakers. Spike one of them with 40 µL of 1 mg/mL arsenic reference solution (final concentration, 2 mg/L).

21.2.3.3 Carefully add 4 mL concentrated HCl to each beaker.

21.2.3.4 In a hood, heat the solutions to a gentle boil for approximately 1 hour. Add 10% HCl as necessary to maintain the original volume.

21.2.3.5 After 1 hour, remove the copper coils and gently rinse with dH_2O. Compare the negative control and positive control to the unknown for the presence of a gray to black deposit.

21.2.4 Interpretation

21.2.4.1 If the positive and negative control work and the copper coils in the unknown samples are still bright in appearance, then the test can be reported as per section 2.7.

21.2.4.2 If the positive and negative controls work and the copper coils in the unknown samples become gray, black or silvery, then the result is a presumptive positive for the presence of

heavy metals. Since DFS does not have the capability to confirm positive results, the following statement may be reported on the Certificate of Analysis:

21.2.4.2.1 Test results indicate a presumptive positive for the presence of heavy metals (antimony, arsenic, bismuth and mercury) via the Reinsch Test. The samples should be sent to another laboratory for the definitive identification, confirmation and quantitation of heavy metals.

21.3 References

21.3.1 I., Sunshine, Methodology for Analytical Toxicology, CRC Press, Cleveland, OH, 1975.

21.3.2 N. W. Tietz, Fundamentals of Clinical Chemistry, W.B. Saunders, Philadelphia, PA, 1976.

21.3.3 E.C.G. Clarke, Clark's Isolation and Identification of Drugs, The Pharmaceutical Press, London, UK, 1986.

22 ACETAMINOPHEN AND SALICYLATE QUANTITATION AND CONFIRMATION BY HPLC

22.1 Summary

Acetaminophen and salicylic acid are extracted from biological samples by making the samples slightly acidic with sodium acetate buffer and extracting with hexane/ethyl acetate. An aliquot of the extract is analyzed by high performance liquid chromatography-diode array detector (HPLC-DAD).

22.2 Specimen Requirements

0.5 mL blood, fluid or tissue homogenate.

22.3 Reagents and Standards

- Acetaminophen, 1 mg/mL
- Salicylic Acid, 1 mg/mL
- Phenacetin, 1 mg/mL
- Sodium acetate
- Hexane
- Ethyl acetate
- Methanol
- Glacial Acetic Acid

22.4 Solutions, Internal Standard, Calibrators and Controls

22.4.1 Sodium Acetate Buffer - weigh out 8.0 g sodium acetate, transfer to a 1 L volumetric flask and dissolve in approximately 800 mL deionized water. Adjust the pH to 4.5 with glacial acetic acid and qs to volume with deionized water. Store at room temperature for up to one month.

22.4.2 Hexane/Ethyl acetate (50:50, v:v) - Mix 100 mL hexane with 100 mL ethyl acetate

22.4.3 Mobile Phase A (water with 0.2% glacial acetic acid) - In a 500 mL volumetric flask filled with approximately 450 mL of HPLC grade water, add 1 mL of glacial acetic acid and qs to volume with HPLC grade water. Filter before use. Store at room temperature for up to one month.

22.4.4 Drug stock solutions:

If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and qs to volume with methanol. Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free drug, and weigh this amount. Stock solutions are stored capped in a refrigerator and are stable for up to 2 years.

22.4.5 The following are examples of acceptable procedures for the preparation of calibrators. Other quantitative dilutions may be acceptable to achieve similar results:

22.4.5.1 Working acetaminophen standard solution (0.5 mg/mL). Pipet 5 mL of 1 mg/mL acetaminophen stock solution into a 10 mL volumetric flask and qs to volume with methanol.

22.4.5.2 Working salicylic acid standard solution (0.25 mg/mL). Pipet 2.5 mL of 1 mg/mL salicylic acid stock solution into a 10 mL volumetric flask and qs to volume with methanol.

22.4.5.3 Working internal standard solution (50 µg/mL phenacetin): Pipet 500 µL of the 1 mg/mL stock solution of phenacetin into a 10 mL volumetric flask and qs to volume with methanol.

22.4.5.4 To prepare the calibration curve, pipet the following volumes of the 0.5 mg/mL acetaminophen stock solution and/or 0.25 mg/mL salicylic acid stock solution into appropriately labeled 16 x 125 mm screw cap test tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 0.5 mL blank blood to obtain the final concentrations listed below.

Amount of stock solution (µL)	Final concentration of acetaminophen (mg/L)	Final concentration of salicylic acid (mg/L)
400	400	200
200	200	100
100	100	50
60	60	30
40	40	20
20	20	10
10	10	5

22.4.6 Controls

22.4.6.1 Acetaminophen Control. Control may be from an external source or prepared in-house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

22.4.6.2 Salicylic Acid Control. Control may be from an external source or prepared in-house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.

22.4.6.3 Negative control. Blood bank blood or equivalent determined not to contain acetaminophen, salicylic acid or phenacetin.

22.5 Apparatus

22.5.1 Test tubes, round bottom, borosilicate glass with Teflon caps

22.5.2 Test tubes, glass centrifuge, conical bottom

22.5.3 Centrifuge capable of 2000-3000 rpm

22.5.4 Evaporator/concentrator

22.5.5 Vortex mixer

22.5.6 GC autosampler vials with inserts

22.5.7 pH meter

22.5.8 HPLC-DAD

22.5.8.1 HPLC Instrument Conditions. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

22.5.8.1.1 Elution Conditions

Column: Agilent Zorbax C8 150 mm x 4.6 mm x 5 µm

Column temp: 35°C

Solvent A: water with 0.2% glacial acetic acid

Solvent B: methanol

Initial Flow Rate: 0.50 mL/min

Injection vol.: 3 µL with wash vial

Stop time: 10.0 min with 7.0 min post time

Gradient:

0-3.5 minutes	50% B	0.50 mL/min
3.5-4 minutes	50% B	0.70 mL/min
4-9 minutes	50% B	0.70 mL/min
9-10 minutes	50% B	0.50 mL/min

Wavelength: 250 nm, switch to 240 nm at 6.8 min

22.6 Procedure

22.6.1 Label clean screw cap tubes appropriately with calibrators, controls and case sample IDs.

22.6.2 Prepare calibrators and controls.

22.6.3 Add 0.5 mL case specimens to the appropriately labeled tubes.

22.6.4 Add 50 µL of the 50 µg/mL phenacetin internal standard working solution to each tube for a final concentration of 5 mg/L.

22.6.5 Add 1 mL sodium acetate buffer and 3 mL extraction solvent (50:50 hexane/ethyl acetate) to each tube.

22.6.6 Cap and rotate tubes for 30 minutes.

22.6.7 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Transfer organic (upper) layer to appropriately labeled conical bottom test tubes.

22.6.8 Evaporate samples to dryness at approximately 60°C under nitrogen.

22.6.9 Reconstitute samples in 200 µL methanol. Transfer to GC autosampler vials for analysis.

22.7 Quality Control and Reporting

22.7.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte.

22.7.2 The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

22.7.3 See Toxicology Quality Guidelines

22.8 Note

This procedure may also be used for the quantitation and confirmation of other non-steroidal anti-inflammatory drugs (e.g., naproxen) with appropriate calibrators and controls.

22.9 References

23.9.1 Melissa Kennedy, Debbi Sullivan, Robert Steiner, Dwight Flammia and Christina Martinez, in-house development.

23 ETHANOL CONTENT OF ALCOHOLIC BEVERAGES BY HEADSPACE GC

The Virginia Department of Alcoholic Beverage Control (ABC) regulates the sale of alcoholic beverages and enforces the alcoholic beverage control laws within the Commonwealth of Virginia.

The DFS Toxicology Lab tests beverages submitted by law enforcement agencies. Most of these cases involve the investigation of minors in possession of alcohol, open intoxicants in vehicles and illegal sale/distribution of alcohol. These types of cases require the analysis of alcohol content. Any beverage containing greater than or equal to 0.5% ethanol is defined as an alcoholic beverage (Code of Virginia § 4.1-100).

23.1 Summary

An aliquot of sample is diluted semi-automatically with an internal standard (IS) solution (n-propanol) into a glass vial, sealed, and placed in a heated automatic sampler. The concentration of ethanol in a dilute alcoholic beverage is directly proportional to its concentration in the gas phase or headspace. A portion of the resultant headspace vapor above the liquid is automatically injected into a gas chromatograph (GC) equipped with two flame ionization detectors (FID). Ethanol is identified by retention time and its concentration is calculated by comparison to similarly treated aqueous calibrators by using peak heights or areas.

23.2 Specimen Requirements

Approximately 0.5 mL liquid or 0.5 g solid material

23.3 Reagents and Standards

- Absolute ethanol, 200 proof
- N-propanol
- Reference standard ethanol solutions (e.g., National Institute of Standards and Technology (NIST) or NIST traceable)

23.4 Solutions, Internal Standard, Calibrators and Controls

23.4.1 All calibrators and controls shall be stored at 2-8ºC for up to one year.

23.4.2 0.5% v/v n-propanol internal standard. Pipet 5 mL n-propanol into a 1 L volumetric flask and qs to volume with dH_2O.

23.4.3 0.5% v/v ethanol standard. Pipet 500 µL ethanol into a 100 mL volumetric flask and qs to volume with dH_2O.

23.4.4 5% v/v ethanol standard. Pipet 5 mL ethanol into a 100 mL volumetric flask and qs to volume with dH_2O.

23.4.5 25% v/v ethanol standard. Pipet 25 mL ethanol into a 100 mL volumetric flask and qs to volume with dH_2O.

23.4.6 50% v/v ethanol standard. Pipet 50 mL ethanol into a 100 mL volumetric flask and qs to volume with dH_2O.

23.4.7 Controls

23.4.7.1 WQA Control. Red wine (12-14% v/v ethanol) stored in 4 ounce glass bottles (stored at 2-8°C for up to two years).

23.4.7.2 NIST ethanol solutions (7.5% and 31.25% v/v ethanol)

23.4.7.3 Negative control (dH_2O)

23.4.7.4 5% v/v ethanol in-house control. Pipet 5 mL ethanol into a 100 mL volumetric flask and qs to volume with dH_2O.

23.5 Apparatus

23.5.1 Gas chromatograph with data system, two flame ionization detectors and a headspace autosampler

23.5.2 Columns. Restek Rtx®-BAC1 and Rtx®-BAC2, or Agilent DB-ALC1 and Agilent DB-ALC2 capillary columns, or Agilent DB-BAC1 UI and DB-BAC2 UI capillary columns

23.5.3 Glass 20 mL (23 x 75 mm) headspace vials with butyl septa and metal seals

23.5.4 Vial seal crimper

23.5.5 Hamilton Microlab 5000 Series Diluter or equivalent

23.5.6 Glass Test tubes (12 x 75 mm)

23.5.7 Headspace GC-FID parameters. Instrument conditions may be changed to permit improved performance.

23.5.7.1 GC parameters

- Oven 50ºC Isothermal
- Injector 200ºC
- Detector (FID) 300ºC
- Hydrogen flow 30 mL/min
- Air flow 400 mL/min
- Make-up flow 20 mL/min
- Make-up gas helium
- Inlet Split
- Split ratio 50:1
- Split flow 674 mL/min
- Total flow 690 mL/min
- Pressure 8 psi constant pressure mode

23.5.7.2 Headspace autosampler parameters

- Incubation temp 70ºC
- Incubation time 240 seconds
- Syringe temp 80ºC
- Agitator speed 500 rpm
- Fill speed 1000 µL/sec
- Injection speed 1000 µL/sec
- Syringe 2.5 mL headspace

23.6 Procedure

Case samples are prepared and analyzed in duplicate. Calibrators and controls are analyzed singly.

23.6.1 Pour approximately 0.2 mL of calibrator, control or sample into a clean 12 x 75 mm test tube.

23.6.2 Place the diluter delivery tip into the specimen, making sure its tip is below the surface of the sample. Activate the dilutor. At this point, the dilutor draws 0.05 mL of sample into its delivery tube.

23.6.3 Withdraw the tip and wipe it with a Kimwipe/tissue paper.

23.6.4 Direct the delivery tip into the appropriately labeled headspace vial and activate the dilutor. The dilutor will dispense the sample and 0.950 mL of the n-propanol IS solution into the vial.

23.6.5 Flush the dilutor as necessary by activating the dilutor one or more times or rinsing with dH_2O, depending on the viscosity or other nature of the sample. Dispense washings into a waste beaker.

23.6.6 Stopper the headspace vial with the butyl septa. Vortex or manually shake for several seconds and place in the sample rack.

23.6.7 Repeat steps 24.6.1 through 24.6.6 for all calibrators, controls and case samples.

23.6.8 Seal all headspace vials by crimping the metal rings over the butyl septa.

23.6.9 Load headspace vials into the headspace auto sampler.

23.7 Calculation

23.7.1 Ethanol is identified based on relative retention time compared to calibrators. Identification is performed by instrument software. Retention times for both ethanol and internal standard should be within ± 2% of the retention time obtained from the average of the calibrators on both columns.

23.7.2 Concentration is calculated automatically by the software based on linear regression of the 4 point calibration curve based on peak area or peak height measurement.

23.7.3 Solid material concentration is calculated as follows:

$$\text{Chromatogram concentration} \times \frac{0.5\ \text{g}}{\text{weighed amount}} = \text{ethanol concentration \% (w/w)}$$

23.8 Quality Control and Reporting

23.8.1 Calibration check. The method calibration is checked with each day's batch sample analysis. Analyze the 4 calibrators, negative control (dH_2O), burgundy wine QC and NIST QCs. Acceptable tolerance for calibrators and controls is 5% of the target value.

23.8.1.1 If the calibrators and controls satisfy quality control criteria, the method may be used as is.

23.8.1.2 If the calibrators and/or controls do not satisfy quality control criteria, recalibrate the method.

23.8.1.3 If, after recalibration, the calibrators and controls still do not fall within 5% of target concentration, then appropriate measures must be taken to rectify the problem (open or prepare new calibrators/controls, instrument maintenance etc). Document such actions and measure in the BAC instrument log.

23.8.1.4 Samples may not be accepted prior to an acceptable calibration check.

23.8.2 Correlation of determination (r^2). The r^2 value for the linear regression curve must be 0.995 or greater. If not, the instrument must be recalibrated or other appropriate measures taken. The correlation of determination is automatically printed on the calibration table and curves (and a copy should be included with the batch data file).

23.8.3 Carryover. The negative control (dH_2O) is used to check for carryover and is run immediately following the high 50% ethanol calibrator. An acceptable negative control must contain less than 0.1% ethanol w/v.

23.8.3.1 If the negative control is unacceptable, prepare a fresh negative dH2O control and reanalyze immediately after the high calibrator. If, after reanalysis, ethanol is present in the negative control greater than 0.1% w/v, perform instrument maintenance to correct the problem.

23.8.4 Positive controls. The acceptable tolerance for ethanol controls is 5% of target concentration. If any of the controls fall outside of the acceptable tolerance, all positive samples must be repeated. Negative results may

be reported. Document actions and exceptions on the batch worksheet and the instrument log. In general, corrective actions for failed controls may include repeating the batch, recalibrating the instrument, opening new controls or making new calibrators.

23.8.5 Batch sample analysis. Headspace ethanol analysis is performed as a batch analysis. Analyze one control after every 10 injections. All controls within a batch must fall within acceptance tolerance in order to report any positive cases from that batch.

23.8.6 Vial Verification. After the completion of a batch, the identity of each vial in the headspace sampler is verified with the sequence table and the alcohol worksheet. Vial verification is performed by an analyst other than the operator and is documented by initials and date on the batch alcohol worksheet.

23.8.7 Case samples are analyzed in duplicate. Duplicate results must agree within 10%. Otherwise, reanalyze samples in duplicate.

23.8.8 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve. The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve.

23.8.9 Report the average of the duplicates rounded to two (2) significant figures (e.g., 5.4% v/v)

23.9 References

23.9.1 B. Kolb, "Head Space Analysis by Means of the Automated Gas Chromatograph F-40 Mulitfract", Bodenseewerk Perkin-Elmer and Co., Technical Manual #15E.

23.9.2 K. M. Dubowski, "Manual for Analysis of Ethanol in Biological Liquids," Department of Transportation Report No. DOT TSC NHTSA-76-4, Jan 1977.

24 CANNABINOID QUANTITATION AND CONFIRMATION BY LCMSMS

24.1 Summary

Δ9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy- Δ9-tetrahydrocannabinol (THC-COOH, Carboxy-THC) are extracted from biological samples by adding acetic acid and hexane:ethyl acetate (9:1). An aliquot of the extract is quantitated and confirmed by LCMSMS. Although not routine, 11-hydroxy- Δ9-tetrahydrocannabinol (OH-THC) may also be quantitated and confirmed via this method.

24.2 Specimen Requirements

1 mL blood, fluid or tissue homogenate

24.3 Reagents and Standards

- Drug targets and internal standards

Targets	Internal Standards
Δ9-tetrahydrocannabinol (THC)	Δ9-tetrahydrocannabinol-D3
11-nor-9-carboxy- Δ9-tetrahydrocannabinol (Carboxy-THC, THC-COOH)	11-nor-9-carboxy- Δ9-tetrahydrocannabinol-D3
11-hydroxy- Δ9-tetrahydrocannabinol (OH-THC), if performed	11-hydroxy- Δ9-tetrahydrocannabinol-D3, if performed

- Acetic acid, glacial, ACS Plus or higher grade
- n-Hexane, Fisher Optima grade or higher grade
- Ethyl acetate, Fisher Optima grade or higher grade
- HPLC grade water/dH2O
- Acetonitrile, Fisher Optima grade or higher grade
- Formic acid, eluent additive for LC-MS

24.4 Solutions, Internal Standard, Calibrators and Controls

24.4.1 10% Acetic Acid: pipette 50 mL of glacial acetic acid into a 500 mL volumetric flask and qs to volume with dH_2O. Store at room temperature for up to 2 years.

24.4.2 Hexane:Ethyl Acetate (9:1): Add 900 mL hexane into a 1 L graduated cylinder. Add 100 mL of ethyl acetate. Store at room temperature for up to 2 years.

24.4.3 Acetonitrile:Water (50:50): Mix 50 mL acetonitrile with 50 mL HPLC grade water. Store at room temperature for up to 2 years.

24.4.4 Mobile Phase A (H_2O with 0.1% formic acid): add 1 mL of formic acid to 1 L of dH_2O. Store at room temperature for up to one month.

24.4.5 Mobile Phase B (Acetonitrile with 0.1% formic acid): add 1 mL of formic acid to 1 L of acetonitrile. Store at room temperature for up to one month.

24.4.6 Preparation of calibrators

24.4.6.1 Working internal standard solution (1 µg/mL): Pipette 100 µL of the 0.1 mg/mL (or 10 µL of 1.0 mg/mL) stock solution of deuterated standards into a 10 mL volumetric flask and qs to volume with methanol.

24.4.6.2 Working internal standard solution (0.1 µg/mL): Pipette 1 mL of the 1 µg/mL working internal standard solution of deuterated standards into a 10 mL volumetric flask and qs to volume with methanol.

24.4.6.3 Working standard solution (1/5 µg/mL): Pipette 10 µL/50 µL (25 µL/125 µL) of the 1.0 mg/mL stock solution standards (THC, OH-THC/Carboxy-THC) into a 10 mL (25 mL) volumetric flask and qs to volume with methanol. Only prepare in 25 mL volumetric flask if a calibrated volumetric flask is available.

24.4.6.4 Working standard solution (0.1/0.5 µg/mL): Pipette 1 mL of the 1/5 µg/mL working standard solution into a 10 mL volumetric flask and qs to volume with methanol.

24.4.6.5 To prepare the calibration curve, pipette the following volumes of the 1/5 or the 0.1/0.5 µg/mL working cannabinoid standard solution into appropriately labeled 16 x 125 mm screw cap test tubes. Calibrators and controls shall not be dried down under any circumstances (i.e using nitrogen or heat). Add 1 mL blank blood to obtain the final concentrations listed below.

Amount of 1/5 µg/mL stock solution (µL)	Amount of 0.1/0.5 µg/mL stock solution (µL)	Final concentration of cannabinoids (mg/L)
100		0.100/0.500
50		0.050/0.250
25		0.025/0.125
	100	0.010/0.050
	50	0.005/0.025
	25	0.0025/0.0125
	10	0.001/0.005

24.4.7 Controls

24.4.7.1 Cannabinoid Control. Run control from an external source (e.g., UTAK), if available. In-house controls should be prepared using standards from different manufacturers or lot numbers.

24.4.7.2 Negative control. Blood bank blood or equivalent determined not to contain cannabinoids.

24.5 Apparatus

24.5.1 Test tubes, round bottom, borosilicate glass with Teflon caps

24.5.2 Test tubes, conical bottom

24.5.3 Centrifuge capable of 2000-3000 rpm

24.5.4 Evaporator/concentrator

24.5.5 Vortex mixer

24.5.6 GC autosampler vials with inserts

24.5.7 Typical LCMSMS parameters. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

24.5.7.1 LC Parameters

- Column: Poroshell 120 EC-C18, 2.1 x 75 mm, 2.7 µm particle size

- Column Thermostat: 40°C
- Solvent A: H_2O with 0.1% formic acid
- Solvent B: Acetonitrile with 0.1% formic acid
- Initial Flow Rate: 0.50 mL/min
- Injection vol.: 10 µL with 5 second needle wash
- Stop time: 10.5 min
- Gradient:

Initial	40% B
1 minutes	40% B
7 minutes	95% B
10 minutes	95% B
10.5 minutes	40% B
Post time 2 minutes	

24.5.7.2 Typical MS-MS parameters. The following instrument conditions may be modified to adjust or improve sensitivity. Optimizer should be used for each compound to set the fragmentor, collision energy, and cell accelerator voltages.

- MSD Parameters:

Ionization:	ESI
Polarity:	positive
Gas temp:	350°C
Drying Gas:	10.0 L/min
Nebulizer press:	40 psi
Capillary:	4000 V

- Transition Ions
  - Time Segments (TS)
  - TS1 – 0-4 minutes (To Waste)
  - TS2 – 4-6.15 minutes (Carboxy-THC-D3, Carboxy-THC, OH-THC-D3, OH-THC)
  - TS3 – 6.15-8 minutes (THC-D3, THC)
  - TS4- 8-10.5 minutes (To Waste)

Internal Standard	Target	Precursor Ion	Product Ions
THC-COOH-D3		348	330,302
	THC-COOH	345	299,193
OH-THC-D3		334	316,196
	OH-THC	331	313,193
THC-D3		318	196,123
	THC	315	193,123

24.6 Procedure

24.6.1 Note: Urine specimens may be hydrolyzed to remove glucuronide conjugates prior to extraction using the following hydrolysis procedure. A glucuronide positive control should be used to ensure the hydrolysis was effective.

24.6.1.1 Alkaline hydrolysis

Add 40 µL of 10 N NaOH to 1 mL of each urine specimen. The pH should be greater than 10. Cap, vortex and heat at 60°C for 20 minutes. After cooling, add approximately 25 µL of glacial acetic acid or as necessary to neutralize pH.

24.6.2 Prepare calibrators and controls.

24.6.3 Add 1.0 mL case specimens/blank blood to the appropriately labeled tubes.

24.6.4 Add 100 µL of the 0.1 µg/mL internal standard working solution to each tube.

24.6.5 Add 2 mL of water and vortex briefly.

24.6.6 Add 800 µL of 10% acetic acid and vortex.

24.6.7 Add 8.0 mL of 9:1 hexane:ethyl acetate solution.

24.6.8 Cap and rotate tubes for 30 minutes

24.6.9 Centrifuge at approximately 2800 rpm for 15 minutes to achieve separation.

24.6.10 Transfer organic (upper) layer to appropriately labeled tubes.

24.6.11 Evaporate samples to dryness at approximately 40-50°C under nitrogen.

24.6.12 Reconstitute samples in 100 µL of 50:50 acetonitrile:water.

24.6.13 Centrifuge at approximately 2800 rpm for 15 minutes.

24.6.14 Transfer to GC autosampler vials with inserts for LCMSMS analysis.

24.7 Quality Control and Reporting

24.7.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte.

24.7.2 The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

24.7.3 The calibration model for all targets is linear weighted 1/x.

24.7.4 When a target concentration is above the upper limit of quantitation, 1.0 mL of case sample shall be diluted with no more than 3.0 mL of blank blood for a total dilution volume of 4.0 mL. Alternatively, 0.5 mL of case sample may be used for a dilution of 1/2. If less than 0.5 mL of sample is used for analysis, qualitative results may be reported.

24.7.5 Extracted samples are stable for four days after reconstitution.

24.7.6 See Toxicology Quality Guidelines

24.8 References

24.8.1 J.S. Hudson, J.W. Hutchings, P. Friel, in-house development.

24.8.2 Alabama DFS Cannabinoids Method

24.8.3 D.M. Schwope, K.B. Scheidweiler, M.A. Huestis. Direct quantification of cannabinoids and cannabinoid glucuronides in whole blood by liquid chromatography-tandem mass spectrometry. Analytical Bioanalytical Chemistry. 401(4):1273-1283 (2011)

25 BENZODIAZEPINES, ZOLPIDEM, ZOPICLONE AND ZALEPLON QUANTITATION AND CONFIRMATION BY LCMSMS

25.1 Summary

Benzodiazepines, zolpidem, zopiclone, and zaleplon are extracted from biological samples by adding sodium carbonate buffer and extracting with 1-chlorobutane. An aliquot of the extract is quantitated and confirmed by LCMSMS.

Alprazolam, clonazepam, lorazepam, diazepam, nordiazepam, oxazepam, temazepam, and zolpidem are quantitative targets within this method.

7-aminoclonazepam, 7-aminoflunitrazepam, α-hydroxyalprazolam, α-hydroxymidazolam, α-hydroxytriazolam, flunitrazepam, flurazepam, N-desalkylflurazepam, midazolam, phenazepam, triazolam, zopiclone, and zaleplon are qualitative targets that may be quantitated upon toxicologist review.

Etizolam, flubromazolam and flubromazepam are analyzed qualitatively only.

Chlordiazepoxide is analyzed qualitatively only with this method but can be quantitated using the Chlordiazepoxide method.

25.2 Specimen Requirements

1 mL blood, fluid or tissue homogenate

25.3 Reagents and Standards

- Drug targets and associated internal standards. Note: drugs may be analyzed in combinations or separately.

Targets	Internal Standards
7-aminoclonazepam	7-aminoclonazepam-D_4
7-aminoflunitrazepam	7-aminoclonazepam-D_4
Zopiclone	Zopiclone-D_4
Zolpidem	Zolpidem-D_6
Zaleplon	Zolpidem-D_6
Chlordiazepoxide	Diazepam-D_5
Flurazepam	Diazepam-D_5
Nordiazepam	Diazepam-D_5
n-desalkylflurazepam	Diazepam-D_5
Phenazepam	Diazepam-D_5
Diazepam	Diazepam-D_5
α-hydroxyalprazolam	α-hydroxyalprazolam-D_5
α-hydroxymidazolam	α-hydroxyalprazolam-D_5
α-hydroxytriazolam	α-hydroxyalprazolam-D_5
Midazolam	Alprazolam-D_5
Alprazolam	Alprazolam-D_5
Triazolam	Alprazolam-D_5
Oxazepam	Oxazepam-D_5
Lorazepam	Oxazepam-D_5
Clonazepam	Clonazepam-D_4
Flunitrazepam	Clonazepam-D_4
Temazepam	Temazepam-D_5
Etizolam*	Diazepam-D_5

Flubromazolam*	Alprazolam-D_5
Flubromazepam*	Clonazepam-D_4

*Targets may not be run every time but by customer request or laboratory guidance.

- Sodium carbonate, certified ACS powder
- 1-chlorobutane, HPLC grade
- Acetonitrile, Fisher Optima grade or higher grade
- Type I or LC-MS grade water
- Formic Acid, eluent additive for LC-MS

25.4 Solutions, Internal Standard, Calibrators and Controls

25.4.1 0.2 M Sodium Carbonate: weigh out 10.6 g sodium carbonate, transfer to a 500 mL volumetric flask and qs to volume with dH_2O. Store at room temperature for up to 2 years.

25.4.2 Mobile Phase A (H_2O with 0.1% formic acid): add 1 mL of formic acid to 1 L of Type I or LC-MS grade H_2O. Store at room temperature for up to one month.

25.4.3 Mobile Phase B (Acetonitrile with 0.1% formic acid): add 1 mL of formic acid to 1 L of acetonitrile. Store at room temperature for up to one month.

25.4.4 Drug stock solutions:

If 1 mg/mL commercially prepared stock solutions are not available, prepare 1 mg/mL solutions from powders. Weigh 10 mg of the free drug, transfer to a 10 mL volumetric flask and qs to volume with methanol. Note: If using the salt form, determine the amount of the salt needed to equal 10 mg of the free drug, and weigh this amount. Stock solutions are stored capped in a freezer and are stable for up to 2 years or manufacturer's specifications, whichever is earlier.

25.4.5 Preparation of calibrators.

25.4.5.1 Working standard solution A (0.01 mg/mL): This standard solution is used to prepare calibrators of frequently quantitated targets. Pipette 100 µL of the 1 mg/mL (or 1 mL of 0.1 mg/mL) stock solution into a 10 mL volumetric flask and qs to volume with methanol. Targets include: alprazolam, clonazepam, diazepam, lorazepam, nordiazepam, oxazepam, temazepam, and zolpidem.

25.4.5.2 Working standard solution A (0.001 mg/mL): Pipette 1.0 mL of the 0.01 mg/mL working standard solution A into a 10 mL volumetric flask and qs to volume with methanol.

25.4.5.3 Working standard solution B (0.01 mg/mL): This standard solution is used to prepare calibrators of infrequently quantitated targets. Pipette 100 µL of the 1 mg/mL (or 1 mL of 0.1 mg/mL) stock solution into a 10 mL volumetric flask and qs to volume with methanol. Targets may include: n-desalkylflurazepam, midazolam, phenazepam, and triazolam.

25.4.5.4 Working standard solution B (0.001 mg/mL): Pipette 1.0 mL of the 0.01 mg/mL working standard solution B into a 10 mL volumetric flask and qs to volume with methanol.

25.4.5.5 Working standard solution C (0.01 mg/mL): This standard solution is used in the method to act as a qualitative one-point calibrator. Pipette 100 µL of the 1 mg/mL (or 1 mL of 0.1 mg/mL) stock solution into a 10 mL volumetric flask and qs to volume with methanol. Targets include: 7-aminoclonazepam, 7-aminoflunitrazepam, α-hydroxyalprazolam, α-hydroxymidazolam, α-hydroxytriazolam, n-desalkylflurazepam, chlordiazepoxide, flunitrazepam, flurazepam, midazolam, phenazepam, triazolam, etizolam, flubromazepam, zaleplon, zopiclone, and flubromazolam.

25.4.5.6 Stock internal standard solution (0.01 mg/mL): Pipette 100 µL of the 1 mg/mL (or 1 mL of 0.1 mg/mL) stock solution of deuterated standards into a 10 mL volumetric flask and qs to volume with methanol.

25.4.5.7 Working internal standard solution (0.001 mg/mL): Pipette 1.0 mL of the 0.01 mg/mL stock internal standard solution into a 10 mL volumetric flask and qs to volume with methanol.

25.4.5.8 To prepare calibration curve A or B, pipette the following volumes of the 0.01 mg/mL and 0.001 mg/mL working standard solution A or B into appropriately labeled 16 x 125 mm screw cap test tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 1 mL blank blood to obtain the final concentrations listed below.

Calibrators shall be divided into three calibrator solutions. Calibrator working standard A will be spiked quantitatively for every analysis. In addition, calibrator working standard C will be spiked and analyzed as a qualitative one-point calibrator. In the event that a target from working standard C is positive, a separate extraction shall be spiked using working standard B.

Amount of 0.01 mg/mL stock solution (µL)	Amount of 0.001 mg/mL stock solution (µL)	Final concentration of benzodiazepines (mg/L)
200		2.0
100		1.0
50		0.5
	100	0.1
	50	0.05
	20	0.02
	10	0.01

Working standard C shall be prepared by pipetting 50 µL of 0.01 mg/mL working standard solution C for a qualitative calibrator.

25.4.6 Controls

25.4.6.1 Benzodiazepine/ZZZ Controls. Controls may be from an external source or prepared in-house using drugs from different manufacturers or lot numbers.

25.4.6.1.1 Due to the quadratic nature of many of the targets, at least three controls, at low medium and high concentrations, must be run across the concentration range in every batch. If the high calibrator is 2 mg/L, a high control between 1 and 2 mg/L must be run.

25.4.6.2 Negative control. Blood bank blood or equivalent determined not to contain benzodiazepines or other targets.

25.5 Apparatus

25.5.1 Test tubes, round bottom, borosilicate glass with Teflon caps

25.5.2 Test tubes, conical bottom

25.5.3 Centrifuge capable of 2000-3000 rpm

25.5.4 Evaporator/concentrator

25.5.5 Vortex mixer

25.5.6 GC autosampler vials with inserts

25.5.7 Typical LCMSMS parameters. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

25.5.7.1 LC Parameters

- Column: Poroshell 120 EC-C18, 2.1 x 75 mm, 2.7 µm particle size
- Column Thermostat: 35°C
- Mobile Phase A: H_2O with 0.1% formic acid
- Mobile Phase B: Acetonitrile with 0.1% formic acid
- Initial Flow Rate: 0.50 mL/min
- Injection vol.: 3 µL with 5 second needle wash
- Stop time: 11 min
- Gradient:

Initial	10% B
4 minutes	30% B
8 minutes	40% B
8.5 minutes	95% B
10.5 minutes	95% B
11 minutes	10% B
Post Time	1.5 min

25.5.7.2 Typical MS-MS parameters. The following instrument conditions may be modified to adjust or improve sensitivity. Optimizer should be used for each compound to set the fragmentor, collision energy, and cell accelerator voltages.

- MSD Parameters:

Ionization:	ESI
Polarity:	positive
Gas temp:	350°C
Drying Gas:	10.0 L/min
Nebulizer press:	50 psi
Capillary:	4000 V

- Transition Ions

Internal Standard	Target	Precursor Ion	Product Ions
7-aminoclonazepam D4		290	121,77
	7-aminoclonazepam	286	121,77
	7-aminoflunitrazepam	284	135,77
zopiclone D4		393	245,217
	Zopiclone	389	245,217
zolpidem D6		314	263,235
	Zolpidem	308	263,235
	Zaleplon	306	264,236
diazepam D5		290	198,154
	Chlordiazepoxide	300	227,89
	Flurazepam	388	317,315
	Nordiazepam	271	165,140
	Diazepam	285	193,91
	n-desalkylflurazepam	289	140,226
	Phenazepam	351	206,179

	Etizolam	343.1	314, 289
alpha-hydroxyalprazolam D5		330	302,210
	alpha-hydroxyalprazolam	325	216,205
	alpha-hydroxymidazolam	342	324,168
	alpha-hydroxytriazolam	359	239,176
alprazolam D5		314	286,210
	Alprazolam	309	205,151
	Triazolam	343	315,308
	Midazolam	326	291,249
	Flubromazolam	371	292,342.9
oxazepam D5		292	274,246
	Oxazepam	287	269,241
	Lorazepam	321	303,229
clonazepam D4		320	274,218
	Clonazepam	316	270,214
	Flunitrazepam	314	268,239
	Flubromazepam	333	226,104
temazepam D5		306	288,260
	Temazepam	301	283,255

25.6 Procedure

25.6.1 Note: Urine specimens may be hydrolyzed to remove glucuronide conjugates prior to extraction using one of the following hydrolysis procedures. A glucuronide positive control should be used to ensure the hydrolysis was effective.

25.6.1.1 Enzyme hydrolysis

Add 5000 Fishman units of β-glucuronidase to each mL of urine. Perform hydrolysis as recommended by the supplier based on the source of β-glucoronidase (e.g., 5000 F units/mL Patella vulgata in 100 mM acetate buffer (pH 5.0) hydrolyzed at 65°C for 3 hours).

25.6.1.2 Alkaline hydrolysis

Add 80 µL of 10 N NaOH to 2 mL of each urine specimen. The pH should be greater than 10. Cap, vortex and heat at 60°C for 20 minutes. After cooling, add 50 µL of glacial acetic acid and acetate buffer as necessary to neutralize pH.

25.6.2 Label clean screw cap tubes appropriately with calibrators, controls and case sample IDs.

25.6.3 Prepare calibrators and controls.

25.6.4 Add 1.0 mL case specimens to the appropriately labeled tubes.

25.6.5 Add 100 µL of the 0.001 mg/mL internal standard working solution to each tube and vortex.

25.6.6 Add 1 mL sodium carbonate and 6 mL 1-chlorobutane to each tube.

25.6.7 Cap and rotate tubes for 30 minutes.

25.6.8 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. Transfer organic (upper) layer to appropriately labeled tubes.

25.6.9 Evaporate samples to dryness at approximately 50°C under nitrogen.

25.6.10 Reconstitute samples in 200 µL methanol. Transfer to GC autosampler vials with inserts for LCMSMS analysis.

25.7 Quality Control and Reporting

25.7.1 For quantitated targets, the LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte.

25.7.2 For quantitated targets, the upper limit of quantitation (ULOQ) for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

25.7.3 Qualitative targets (i.e., 7-aminoclonazepam, 7-aminoflunitrazepam, α-hydroxyalprazolam, α-hydroxymidazolam, α-hydroxytriazolam, n-desalkylflurazepam, chlordiazepoxide, flunitrazepam, flurazepam, midazolam, phenazepam, triazolam, flubromazolam and flubromazepam) are compared to the qualitative (one-point) calibrator prepared from working standard solution C.

If present, n-desalkylflurazepam, midazolam, phenazepam, and triazolam may be quantitatively assessed using a calibration curve prepared from working standard solution B. Chlordiazepoxide may be quantitatively assessed (see ¶ 15).

25.7.4 The calibration model for benzodiazepines is weighted quadratic whereas the calibration model for zolpidem, zopiclone and zaleplon is weighted linear. Samples with a concentration greater than 1 mg/L for a target with a quadratic fit must be repeated if the high positive control is outside of acceptable limits.

25.7.5 When a target concentration is above the upper limit of quantitation, 1.0 mL of case sample shall be diluted with no more than 3.0 mL of blank blood for a total dilution volume of 4.0 mL. Alternatively, 0.5 mL of case sample may be used for a dilution of 1/2. If less than 0.5 mL of sample is used for analysis, only qualitative results may be reported.

25.7.6 Extracted samples are stable for seven days with the exception of diazepam and triazolam which are only stable for six days after reconstitution.

25.7.7 See Toxicology Quality Guidelines.

25.8 References

25.8.1 J.S. Hudson, J.W. Hutchings, P. Friel, in-house development

25.8.2 Remane D, Meyer MR, Wissenbach DK and Maurer HH. “Ultra high performance liquid chromatographic-tandem mass spectrometric multi-analyte procedure for target screening and quantification in human blood plasma: validation and application for 31 neuroleptics, 28 benzodiazepines, and Z-drugs”, *Anal Bioanal Chem*., 2011, 401(4), pp. 1341-52.

26 AMPHETAMINES, PHENTERMINE AND DESIGNER STIMULANTS QUANTITATION AND CONFIRMATION BY LCMSMS

26.1 Summary

Biological samples are made basic with trisodium phosphate buffer and extracted with 6 mL of n-butylchloride. The organic layer is dried down and reconstituted in mobile phase and injected into the LCMSMS. Drug targets may be analyzed in different combinations or separately as needed.

26.2 Specimen Requirements

1 mL of whole blood, bodily fluids, or tissue homogenate

26.3 Reagents and Standards

- Drug targets and associated internal standards

Targets	Internal Standards
Methcathinone*	Mephedrone-D_3
Pseudoephedrine*	Pseudoephedrine-D_3
Methylone*	Methylone-D_3
Amphetamine	Amphetamine-D_{11}
Methamphetamine	Methamphetamine-D_{11}
MDA	MDA-D_5
Methedrone*	Mephedrone-D_3
MDMA	MDMA-D_5
Phentermine*	Methamphetamine-D_{11}
Mephedrone*	Mephedrone-D_3
Alpha-PVP*	Mephedrone-D_3
MDPV*	Mephedrone-D_3
Bupropion*	Mephedrone-D_3
Ethylone*	Methylone-D_3

*Targets may not be run every time but by customer request or laboratory guidance.

- Trisodium phosphate (Na_3PO_4), ACS powder
- 1-chlorobutane, HPLC grade
- Hydrochloric acid, Optima grade
- 2-Propanol, HPLC grade
- Formic Acid, eluent additive ~98%
- Type I or LC-MS grade water
- Methanol, HPLC grade or higher
- Acetonitrile, Optima grade or higher

26.4 Solutions, Internal Standards, Calibrators and Controls

26.4.1 0.2% Hydrochloric acid in 2-propanol: add 1 mL of concentrated HCl (12 N) into 500 mL of 2-propanol. Store at room temperature for up to one month.

26.4.2 Mobile Phase A (H_2O with 0.1% formic acid): add 1 mL of formic acid to 1 L of Type I or LC-MS grade H_2O. Store at room temperature for up to one month.

26.4.3 Mobile Phase B (Acetonitrile with 0.1% formic acid): add 1 mL of formic acid to 1 L of acetonitrile. Store at room temperature for up to one month.

26.4.4 Saturated trisodium phosphate buffer. Add trisodium phosphate to dH_2O until no more dissolves after vigorous shaking. Store at room temperature for up to 2 years.

26.4.5 Working Solution A (10 µg/mL): Add 100 µL of 1.0 mg/mL standard to a 10 mL volumetric flask and qs to volume with acetonitrile.

26.4.6 Working Solution B (1.0 µg/mL): Pipette 1.0 mL Working Solution A into a 10 mL volumetric flask and qs to volume with acetonitrile.

26.4.7 Working Internal Standard (1 µg/mL): Pipette 10 µL of 1.0 mg/mL (or 100 µL of 0.1 mg/mL) internal standard into 10 mL volumetric flask and qs to volume with acetonitrile.

26.4.8 To prepare the calibration curve, add the following volumes of standard solution into 16 x 125 mm screw cap test tubes. Calibrators and controls shall not be dried down under any circumstances (i.e., using nitrogen or heat). Add 1 mL blank blood to each tube to obtain the final concentrations listed below.

Amount of 10 µg/mL stock solution (µL)	Amount of 1.0 µg/mL stock solution (µL)	Final concentration (mg/L)
200		2.00
100		1.00
50		0.50
25		0.25
	100	0.10
	50	0.05
	20	0.02
	10	0.01

26.4.9 Controls

26.4.9.1 Negative control blood: blood bank blood or equivalent determined not to contain target compounds.

26.4.9.2 In-house control is prepared from a different lot number or different manufacturer of standard.

26.4.9.2.1 Due to the quadratic nature of many of the targets, at least three controls, at low, medium and high concentrations, must be run across the concentration range in every batch. A high control must be run within 1 – 2 mg/L

26.4.9.3 Commercial whole blood control (UTAK or other commercial vendor).

26.5 Apparatus

26.5.1 Test tubes, round bottom, screw cap tubes, borosilicate glass with Teflon caps

26.5.2 Test tubes, conical bottom

26.5.3 Centrifuge capable of 2,000 – 3,000 rpm

26.5.4 Vortex mixer

26.5.5 Test tube rotator

26.5.6 GC autosampler vials and inserts

26.5.7 Typical LCMSMS parameters. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

26.5.8 LC Parameters

- Column: Poroshell 120 EC-C18, 2.1 x 75 mm, 2.7 µm particle size
- Column Thermostat: 50 °C
- Mobile Phase A: H_2O with 0.1% formic acid
- Mobile Phase B: Acetonitrile with 0.1% formic acid
- Initial Flow Rate: 0.5 mL/min
- Injection vol.: 2 µL with 6 second needle wash
- Stop time: 9 min
- Gradient:

Initial	2% B
2 minutes	5% B
4 minutes	10% B
6 minutes	30% B
7 minutes	90% B
8.5 minutes	90% B
9 minutes	2% B
Post time	1 minute

26.5.9 Typical MS-MS parameters. The following instrument conditions may be modified to adjust or improve sensitivity. Optimizer should be used for each compound to set the fragmentor, collision energy and cell accelerator voltages.

- MSD Parameters:

Ionization:	ESI
Polarity:	positive
Gas temp:	350 °C
Drying Gas:	10.0 L/min
Nebulizer pressure:	45 psi
Capillary:	3000 V

- Transition Ions

Internal Standard	Target	Precursor Ion	Product Ions
	α-PVP	232.2	126.1, 91
	Amphetamine	136.1	119.1, 91.1
Amphetamine-D_{11}		147.2	130.1, 98.1
	MDA	180.1	163.1, 105.1
MDA-D_5		185.1	168.1, 110.1
	MDMA	194.1	163.1, 105.1
MDMA-D_5		199.1	165.1, 107.1
	Bupropion	240	184,166
	MDPV	276.3	135, 126
	Mephedrone	178.3	160, 144
Mephedrone-D_3		181.3	163, 148
	Methamphetamine	150.1	119.1, 91.1
Methamphetamine-D_{11}		161.2	127.1, 97.1
	Methcathinone	164.2	146, 130
	Methedrone	194.2	176, 161
	Methylone	208.2	190, 132
	Ethylone	222.1	174, 146
Methylone-D_3		211.2	163, 135
	Phentermine	150.1	91.1, 65.1
	Pseudoephedrine	166.1	148.1, 133.1
Pseudoephedrine-D_3		169.1	151.1, 115

26.6 Procedure

26.6.1 Label clean screw cap tubes appropriately with calibrators, controls, and case sample IDs.

26.6.2 Prepare calibrators and controls.

26.6.3 Add 1 mL of case specimens to the appropriately labeled tubes. Note: since this procedure may be used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case as appropriate. One will serve as a screen and the second as a confirmation.

26.6.4 Add 100 µL of 1 µg/mL internal standard working solution to each tube and vortex.

26.6.5 Add 2 mL saturated trisodium phosphate buffer to each tube. Vortex briefly.

26.6.6 Add 6 mL 1-chlorobutane to each tube.

26.6.7 Cap and rotate for 15 minutes at slow speed.

26.6.8 Centrifuge at approximately 2500 rpm for 15 minutes to achieve separation. If emulsion or suspension forms, knock it down with a wooden stick and centrifuge again.

26.6.9 Transfer upper layer (1-chlorobutane) to clean, screw-cap tube.

26.6.10 Add 100 µL of 0.2% HCl in 2-propanol to each tube and evaporate samples to dryness at approximately 40°C.

26.6.11 Reconstitute in 200 µL of 0.1% formic acid in water.

26.6.12 Inject into LCMSMS

26.7 Quality Control and Reporting

26.7.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte.

26.7.2 The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

26.7.3 The following dilution factors may be utilized for this analysis:

Targets that can be diluted up to 1:20 (0.05 mL sample and 0.95 mL blank blood or 1.0 mL sample and 19.0 mL blank blood)
Methcathinone
Ephedrine/Pseuodephedrine
Methylone
Amphetamine
Methamphetamine
MDA
Methedrone
MDMA
Phentermine
Mephedrone
α-PVP

Targets that can be diluted up to 1:5 (0.2 mL sample and 0.8 mL blank blood or 1.0 mL sample and 4.0 mL blank blood)
Bupropion

Targets that can be diluted up to 1:2 (0.5 mL sample and 0.5 mL blank blood or 1.0 mL sample and 1.0 mL blank blood)
MDPV

26.7.4 The calibration model for all targets is weighted quadratic except methcathinone, pseudoephedrine, methylone, and mephedrone which is weighted linear. Samples with a concentration greater than 1 mg/L for a target with a quadratic fit must be repeated if the high positive control is outside of acceptable limits.

26.7.5 Extracted samples are stable for seven days with the exception of MDA (five days) and methcathinone (three days) after reconstitution.

26.7.6 See Toxicology Quality Guidelines

26.7.7 Positive, confirmed results for pseudoephedrine/ephedrine shall be reported as "Pseudoephedrine/ephedrine."

26.7.8 Pseudoephedrine can be a known precursor to the production of methamphetamine therefore pseudoephedrine may be reported from one analysis if methamphetamine is present. If pseudoephedrine is present without methamphetamine, it needs to be analyzed and detected twice to be reported.

26.8 References

26.8.1 "Amphetamine and Methamphetamine" by Wayne Harrington, Methodology of Analytical Toxicology by Irving Sunshine (Ed), CRC Press, 1975.

26.8.2 R.L. Wagner, J.S. Hudson, J.W. Hutchings, and P. Friel, in-house development.

27 ANTI-EPILEPTIC DRUGS QUANTITATION AND CONFIRMATION BY LCMSMS

27.1 Summary

Anti-epileptic drugs (AEDs) are extracted from biological samples using a methanol precipitation. An aliquot of the extract is quantitated and confirmed by LCMSMS. Drug targets may be analyzed in different combinations or separately as needed.

27.2 Specimen Requirements

0.2 mL blood, fluid or tissue homogenate

27.3 Reagents and Standards

- Drug targets and associated internal standards

Target	Internal Standard
Gabapentin	Gabapentin-D_{10}
Levetiracetam	Levetiracetam-D_6
Lamotrigine	Levetiracetam –D_6
Zonisamide	Topiramate-D_{12}
10,11-dihydro-10-hydroxycarbazepine (Oxcarbazepine Metabolite)	Carbamazepine-D_{10}
Oxcarbazepine	Carbamazepine-D_{10}
Topiramate	Topiramate-D_{12}
Carbamazepine	Carbamazepine-D_{10}
Phenytoin	Phenytoin-D_{10}
Pregabalin	Gabapentin-D_{10}
Lacosamide	Gabapentin-D_{10}

- Methanol, HPLC grade or higher
- Acetonitrile, Fisher Optima grade or higher
- Formic Acid, eluent additive for LC-MS
- Type I or LC-MS grade water
- Ammonium Acetate

27.4 Solutions, Internal Standard, Calibrators and Controls

27.4.1 Mobile Phase A (H_2O with 5 mM Ammonium Acetate): add approximately 0.385 grams of ammonium acetate to 1.0 L of dH_2O. Store at room temperature for up to one month.

27.4.2 Mobile Phase B (Acetonitrile with 0.1% formic acid): add 1.0 mL of formic acid to 1.0 L of acetonitrile. Store at room temperature for up to one month.

27.4.3 Preparation of calibrators.

27.4.3.1 Working internal standard solution (10 µg/mL): Pipette 1.0 mL of the 0.1 mg/mL (or 100 µL of 1.0 mg/mL) stock solution of deuterated standards into a 10 mL volumetric flask and qs to volume with methanol.

27.4.3.2 Working standard solution (100 µg/mL): Pipette 1.0 mL of the 1.0 mg/mL stock solution into a 10 mL volumetric flask and qs to volume with methanol.

27.4.3.2.1 Alternative: (50 µg/mL): Pipette 500 µL of the 1.0 mg/mL stock solution into a 10 mL volumetric flask and qs to volume with methanol.

27.4.3.3 Working standard solution (10 µg/mL): Pipette 1.0 mL of the 100 µg/mL working standard solution into a 10 mL volumetric flask and qs to volume with methanol.

27.4.3.3.1 Alternative: (5 µg/mL): Pipette 1.0mL of the 50 µg/mL working standard solution into a 10 mL volumetric flask and qs to volume with methanol.

27.4.3.4 To prepare the calibration curve, pipette the following volumes of the 100 µg/mL and 10 µg/mL (50 mg/mL and 5 ug/mL, in parentheses in table) working standard solutions into appropriately labeled 16 x 100 mm or 16 x 125 mm screw cap test tubes. To eliminate a solvent effect, calibrators and controls shall be dried under nitrogen prior to the addition of blank blood. Add 0.2 mL blank blood to obtain the final concentrations listed below.

Amount of 100 µg/mL (50 µg/mL) stock solution (µL)	Amount of 10 µg/mL (5 µg/mL) stock solution (µL)	Final concentration of AEDs (mg/L)
80 (160)		40
60 (120)		30
40 (80)		20
20 (40)		10
	100 (200)	5
	50 (100)	2.5
	20 (40)	1

27.4.4 **Controls**

27.4.4.5 AED Controls. Controls may be from an external source or prepared in-house using standards from different manufacturers or lot numbers.

27.4.4.5.1 Due to the quadratic nature of many of the targets, at least three controls, at low medium and high concentrations, must be run across the concentration range in every batch. If the high calibrator is 40 mg/L, a high control must be run within 30 – 40 mg/L.

27.4.4.6 Negative control. Blood bank blood or equivalent determined not to contain AEDs or other targets.

27.5 Apparatus

27.5.1 Test tubes, round bottom, borosilicate glass with Teflon caps

27.5.2 Centrifuge capable of 2000-3000 rpm

27.5.3 Evaporator/concentrator

27.5.4 Vortex mixer

27.5.5 GC autosampler vials with inserts

27.5.6 LCMSMS parameters. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

27.5.6.7 LC Parameters

- Column: Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm particle size
- Column Thermostat: 40 °C
- Mobile Phase A: H_2O with 5 mM Ammonium Acetate

- Mobile Phase B: Acetonitrile with 0.1% formic acid
- Initial Flow Rate: 0.50 mL/min
- Injection vol.: 5.0 µL with 5 second needle wash
- Stop time: 11 min
- Gradient:

Initial	10% B
1 minutes	20% B
8 minutes	60% B
9.5 minutes	95% B
10 minutes	95% B
11 minutes	10% B
Post time	2 minutes

27.5.6.8 MS-MS parameters. The following instrument conditions may be modified to adjust or improve sensitivity. Optimizer should be used for each compound to set the fragmentor, collision energy, and cell accelerator voltages.

- MSD Parameters:

Ionization:	ESI
Polarity:	Positive
Gas temp:	350 °C
Drying Gas:	10.0 L/min
Nebulizer press:	40 psi
Capillary	4000 V

- Transition Ions

Internal Standard	Target	Precursor Ion	Product Ions	RT (approx.)
	Gabapentin	172	154, 95	1.8
	Lacosamide	251	108, 91	3.77
	Pregabalin	160	142, 55	1.91
Gabapentin-D10		182	164, 147	1.8
	Levetiracetam	171	126, 69	2
Levetiracetam-D6		177	160, 132	2
	Lamotrigine	256	159, 145	3.5
	Zonisamide	213	132, 77	3.75
	10,11-dihydro-10-hydroxycarbazepine (Oxcarbazepine Metabolite)	255	237, 194	4.1
	Oxcarbazepine	253	236, 180	4.9
	Topiramate	357	264, 184	5.3
Topiramate-D12		369	270, 190	5.3
	Carbamazepine	237	194, 179	6
Carbamazepine-D10		247	204, 202	6
	Phenytoin	253	182, 104	6
Phenytoin-D10		263	192, 109	6

27.6 Procedure

27.6.1 Label clean screw cap centrifuge tubes appropriately with calibrators, controls and case sample IDs.

27.6.2 Prepare calibrators and controls. To eliminate a solvent effect, calibrators and controls shall be dried under nitrogen prior to the addition of blank blood.

27.6.3 Add 0.2 mL case specimens/blank blood to the appropriately labeled tubes. Note: since this procedure is used for screening and confirmation, it is recommended to analyze two different aliquots (or tissues) with each case as appropriate. One will serve as a screen and the second as a confirmation.

27.6.4 Add 100 µL of the 10 µg/mL internal standard working solution to each tube.

27.6.5 Vortex briefly.

27.6.6 Add 1.0 mL of methanol to each tube and vortex for 15-30 seconds. Ensure that the blood in the bottom of the tube mixes thoroughly with the methanol.

27.6.7 Centrifuge the tubes for 15 minutes at 3000 rpm.

27.6.8 Transfer approximately 150 µL of the upper methanol phase into labeled autosampler vials. Avoid transferring any of the precipitate into the autosampler vial.

27.7 Quality Control and Reporting

27.7.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte.

27.7.2 The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

27.7.3 Dilutions of case samples with target concentrations greater than the ULOQ. Dilutions are listed as 1/2 which is 0.2 mL of case sample diluted with 0.2 mL of blank blood, 1/5 which is 0.2 mL of case sample diluted with 0.8 mL of blank blood, 1/10 which is 0.2 mL of case sample diluted with 1.8 mL of blank blood, 1/20 which is 0.2mL of case sample diluted with 3.8mL of blank blood. After dilution, 0.2 mL of diluted sample is used for extraction.

The following targets can be diluted by a factor of 1/20
Lacosamide
Pregabalin

The following targets can be diluted by a factor of 1/10
Gabapentin
Levetiracetam
Oxcarbazepine Metabolite
Topiramate
Carbamazepine

The following targets can be diluted by a factor of 1/5
Lamotrigine

The following targets can be diluted by a factor of ½
Zonisamide
Oxcarbazepine
Phenytoin

27.7.4 The calibration model for all targets is weighted quadratic except for phenytoin and levetiracetam which is weighted linear. Samples with a concentration greater than 30 mg/L for a target with a quadratic fit must be repeated if the high positive control is outside of acceptable limits.

27.7.5 Extracted samples are stable for seven days with the exception of phenytoin (four days), oxcarbazepine metabolite (five days), and carbamazepine (six days) following reconstitution.

27.7.6 See Toxicology Quality Guidelines

27.8 References

27.8.1 R.L. Wagner, J.S. Hudson, J.W. Hutchings, P. Friel, in-house development.

27.8.2 Daniel Dietmann, Uta Juergas, Bernhard J. Steinhoff, Lea Bonnington, Juergen Wendt; *Simultaneous Analysis of Newer Antiepiliptic Drugs by Rapid Resolution LC/Triple Quadrupole Mass Spectrometry*, ASMS 2008; Agilent Technologies.

28 OPIOID, COCAINE, BENZOYLECGONINE AND COCAETHYLENE QUANTITATION AND CONFIRMATION BY LCMSMS

28.1 Summary

Opioids, cocaine, cocaethylene, and benzoylecgonine are extracted from biological samples using an acetonitrile precipitation. An aliquot of the extract is quantitated and confirmed by LCMSMS. Drug targets may be analyzed in different combinations or separately as needed.

Fentanyl derivatives are extracted using the above stated process and are qualitatively analyzed by LCMSMS using a different acquisition method.

Method title may be abbreviated as: OpiCoc, opicoc, Opi-Coc, opi-coc, Opicoc (or similar)

28.2 Specimen Requirements

1.0 mL of blood, fluid or tissue homogenate (For fentanyl derivatives, use caution in interpretation for tissue homogenates due to potential ionization suppression for targets and internal standard.)

28.3 Reagents and Standards

- Drug targets and associated internal standards (OpiCoc)

Targets	Internal Standards
Morphine	Morphine-D_3
Oxymorphone	Oxymorphone-D_3
Hydromorphone	Hydromorphone-D_3
Codeine	Codeine-D_3
Oxycodone	Oxycodone-D_3
6-Monoacetylmorphine	6-Monoacetylmorphine-D_3
Hydrocodone	Hydrocodone-D_3
Benzoylecgonine	Benzoylecgonine-D_3
Tramadol	Tramadol-^{13}C-D_3
Cocaine	Cocaine-D_3
Meperidine	Meperidine-D_4
Acetyl Fentanyl	Fentanyl-D_5
Cocaethylene	Cocaine-D_3
Fentanyl	Fentanyl-D_5
Methadone	Methadone-D_3

- Fentanyl derivative targets and associated internal standards

Targets	Internal Standards
3-Fluorofentanyl	Fentanyl-D_5
4-Methoxybutyrylfentanyl	
Acetylfentanyl	
Acrylfentanyl	
alpha-Methylacetylfentanyl	
alpha-Methylfentanyl	
Benzodioxolefentanyl	
beta-Hydroxythiofentanyl	
Butyrylfentanyl	
Carfentanil	
cis-3-Methylfentanyl	
Cyclopropylfentanyl	

Despropionylfentanyl
Fentanyl
Furanylfentanyl
meta-Fluorobutyrylfentanyl
meta-Fluorofentanyl
meta-Fluoroisobutyrylfentanyl
Methoxyacetylfentanyl
Ocfentanil
ortho-Fluoroacrylfentanyl
ortho-Fluorobutyrylfentanyl
ortho-Fluorofentanyl
ortho-Fluoroisobutyrylfentanyl
para-Fluoroacrylfentanyl
para-Fluorobutyrylfentanyl
para-Fluorofentanyl
para-Fluoroisobutyrylfentanyl
Phenylfentanyl
Tetrahydrofuranfentanyl
trans-3-Methylfentanyl
U-47700
U-49900
Valerylfentanyl

- LCMS grade ammonium formate
- Formic acid, eluent additive ~98%
- Type I or LCMS grade water
- Acetonitrile, Optima grade or higher
- Methanol, Optima grade or higher

28.4 Solutions, Internal Standard, Calibrators and Controls

28.4.1 Mobile Phase A (H_2O with 0.01% formic acid and 6 mM ammonium formate): add 100 µL of formic acid and approximately 0.385 grams of ammonium formate to 1 L of Type I or LCMS grade H_2O. Store at room temperature for up to one month.

28.4.2 Mobile Phase B

28.4.2.1 OpiCoc: (Acetonitrile with 0.01% formic acid): add 100 µL of formic acid to 1 L of acetonitrile (Optima grade or higher). Store at room temperature for up to one month.

28.4.2.2 Fentanyl Derivatives: (Methanol with 0.01% formic acid): add 100 µL of formic acid to 1 L of methanol (Optima grade or higher). Store are room temperature for up to one month.

28.4.3 Working Calibrator 1-A (1.0/10.0 mg/L): Pipette **10.0 µL** of the 1.0 mg/mL stock solution **(oxymorphone, hydromorphone, 6-monoacetyl morphine, acetyl fentanyl and fentanyl)** into a 10.0 mL volumetric flask. Pipette **100.0 µL** of the 1.0 mg/mL stock solution **(benzoylecgonine, meperidine, tramadol, and methadone)** into the same 10.0 mL volumetric flask and qs to volume with acetonitrile.

28.4.4 Working Calibrator 1-B (0.1/1.0 mg/L): Pipette 1.0 mL of 1.0/10.0 mg/L working calibrator 1-A into 10.0 mL volumetric flask and qs to volume with acetonitrile.

28.4.5 Working Calibrator 2-A (10.0 mg/L): Pipette **100.0 µL** of 1.0 mg/mL stock solution **(morphine, codeine, oxycodone, hydrocodone, cocaethylene, and cocaine)** into a 10.0 mL volumetric flask and qs to volume with acetonitrile.

28.4.6 Working Calibrator 2-B (1.0 mg/L): Pipette 1.0 mL of 10.0 mg/L working calibrator 2-A into 10.0 mL volumetric flask and qs to volume with acetonitrile.

28.4.7 Working Qualitative Mix A (0.5/1.0 mg/L): Pipette 5 µL of 1.0 mg/mL or 50 µL of 0.1 mg/mL stock solution into a 10.0 mL volumetric flask and qs to volume with methanol (trans-3-methylfentanyl, cis-3-methylfentatnyl, carfentanil). Pipette 10 µL of 1.0 mg/mL or 100 µL of 0.1 mg/mL stock solution into a 10.0 mL volumetric flask and qs to volume with methanol (all other fentanyl analogs).

28.4.8 Working Qualitative Mix B (0.005/0.01 mg/L): Pipette 100 µL of 0.5/1.0 mg/L working qualitative mix A into 10.0 mL volumetric flask and qs to volume with methanol.

28.4.9 Working Internal Standard (1.0/2.5 mg/L): Pipette 10.0 µL of the 1.0 mg/mL stock solution into a 10.0 mL volumetric flask (hydromorphone-D_3, 6-monacetylmorphine-D_3, oxymorphone-D_3, and fentanyl-D_5). Pipette 25.0 µL of the 1.0 mg/mL stock solution into the same 10.0 mL volumetric flask and qs to volume with acetonitrile (morphine-D_3, codeine-D_3, hydrocodone-D_3, oxycodone-D_3, benzoylecgonine-D_3, cocaine-D_3, tramadol-^{13}C-D_3, methadone-D_3, and meperidine-D_4).

28.4.10 Controls

28.4.10.1 Negative control blood: blood blank blood or equivalent determined not to contain target compounds.

28.4.10.2 An example for in-house control preparation (this is provided for convenience, this is not the only required approach to controls): create mixes much like Calibrator 1-A, Cal 1-B, Cal 2-A, and Cal 2-B for the same targets and label them as Control 1-A, Crtl 1-B, Ctrl 2-A, and Ctrl 2-B. These can then be used to spike the following control levels:

Control Concentration (mg/L)	Ctrl 1-A (µL)	Ctrl 1-B (µL)	Ctrl 2-A (µL)	Ctrl 2-B (µL)
0.003/0.03/0.03	-	30	-	30
0.01/0.1/0.1	-	100	-	100
0.15/1.5/0.9	150	-	90	

28.4.10.2.1 Due to the quadratic nature of many of the targets, at least three controls, at low, medium, and high concentration, must be run across the concentration range with every batch. A high control must be run between the two highest spiked calibrator concentrations (e.g 0.15/1.5/0.9 mg/L control level in the above table).

28.4.10.3 Commercial whole blood control (UTAK or other commercial vendor) or methanolic statewide controls (prepared by the research analyst or designee).

28.5 Apparatus

28.5.1 Test tubes, round bottom, screw cap tubes, borosilicate glass with Teflon caps

28.5.2 Test tubes, conical bottom

28.5.3 Centrifuge capable of 2,000-3,000 rpm

28.5.4 Vortex mixer

28.5.5 GC autosampler vials and inserts

28.5.6 Typical LCMSMS parameters. The following instrumental conditions may be modified to adjust or improve separation and sensitivity.

28.5.7 Opioid, cocaine, benzoylecgonine, and cocaethylene quantitative method parameters

28.5.7.1 LC Parameters

- Column: Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm particle size
- Column Thermostat: 60.0 °C
- Mobile Phase A: H_2O with 0.01% formic acid and 6 mM ammonium formate
- Mobile Phase B: Acetonitrile with 0.01% formic acid
- Flow Rate: 0.5 mL/min
- Injection Volume: 6 µL with 20 second needle wash
- Stop Time: 9.00 minutes
- Post Time: 2.00 minutes
- Gradient:

Time (minutes)	Solvent A	Solvent B
0.00	98.0	2.0
3.00	85.0	15.0
5.50	35.0	65.0
7.00	5.0	95.0
8.00	5.0	95.0
9.00	98.0	2.0

28.5.7.2 Typical MSMS parameters

- MSD Parameters:

  Ionization: ESI
  Polarity: Positive
  Gas Temperature: 325 °C
  Nebulizer Pressure: 45 psi
  Capillary: 3500 V
  Drying Gas: 12 L/min

- Transition Ions:

Dynamic MRM Compound Settings						
Compound	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)	Cell Accelerator (V)	Retention Time (min)
Morphine	286.2	165.1	148	46	7	1.765
		152.1		70		
Morphine-D_3	289.2	165.1	128	42	7	1.784
		152.1		74		
Oxymorphone-D_3	305.2	287.2	121	14	7	1.979
		230.1		26		
Oxymorphone	302.1	284.1	121	14	7	1.989
		227.1		26		
Hydromorphone	286.2	185.1	153	30	2	2.242
		157.1		46		
Hydromorphone-D_3	289.2	185.1	148	30	2	2.252
		157.1		46		
Codeine-D_3	303.2	165.1	143	42	7	3.080
		152.0		74		
Codeine	300.2	165.1	137	46	7	3.090
		152.0		74		
Oxycodone-D_3	319.2	301.2	126	14	7	3.428
		244.1		26		

Oxycodone	316.2	298.1	121	14	7	3.454
		241.1		26		
6-Monoacetylmorphine-D_3	331.2	165.1	148	46	2	3.568
		152.1		78		
6-Monoacetylmorphine	328.2	165.1	138	42	2	3.570
		152.1		78		
Hydrocodone-D_3	303.2	199.1	133	30	7	3.690
		128.0		70		
Hydrocodone	300.2	199.1	137	30	7	3.699
		128.0		70		
Benzoylecgonine-D_3	293.1	105.0	133	25	7	4.134
		77.0		45		
Benzoylecgonine	290.1	168.1	118	10	7	4.143
		77.0		45		
Tramadol-^{13}C-D_3	269.2	269.2	105	0	2	4.700
		58.1		16	7	
Tramadol	264.1	264.1	119	10	7	4.703
		58.1	180	10		
Cocaine-D_3	307.2	85.1	133	25	7	4.878
		77.0		45		
Cocaine	304.2	182.1	138	5	7	4.879
		77.0		45		
Meperidine-D_4	252.2	224.2	140	15	7	4.906
		178.1		15		
Meperidine	248.2	220.0	140	15	7	4.907
		174.1		15		
Acetyl Fentanyl	323.0	188.0	135	20	7	5.094
		105.0		36		
Cocaethylene	318.2	196.1	123	5	7	5.170
		82.1		18		
Fentanyl-D_5	342.2	188.2	140	25	7	5.368
		105.1		50		
Fentanyl	337.2	188.2	140	25	7	5.378
		105.1		45		
Methadone	310.2	223.0	112	9	7	5.912
		105.0		10		
Methadone-D_3	313.2	268.1	118	12	7	5.916
		105.0		28		

28.5.8 Fentanyl derivative qualitative method parameters

28.5.8.1 LC Parameters

- Column: Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm particle size
- Column Thermostat: 60.0 °C
- Mobile Phase A: H_2O with 0.01% formic acid and 6 mM ammonium formate
- Mobile Phase B: Methanol with 0.01% formic acid
- Flow Rate: 0.7 mL/min
- Injection Volume: 20 µL with 20 second needle wash
- Stop Time: 17.00 minutes
- Post Time: 2.00 minutes

- Gradient:

Time (minutes)	Mobile Phase A	Mobile Phase B
0.00	85.0	15.0
7.00	75.0	25.0
13.00	65.0	35.0
16.00	25.0	75.0
17.00	2.0	98.0

28.5.8.2 MSMS Parameters

- MSD Parameters:

  Ionization: ESI
  Polarity: Positive
  Gas Temperature: 325 °C
  Nebulizer Pressure: 45 psi
  Capillary: 3500 V
  Drying Gas 12 L/min

- Transition Ions: (Note: Target MRMs for all listed compounds may not be removed from the data acquisition method without Program Manager approval due to changes in sensitivity for isomeric compounds. Target transitions are in bold font, qualifier transition is in normal font.)

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)	Cell Accelerator Voltage (V)	Retention Time (min)	Delta Retention Time (min)
Methoxyacetylfentanyl	**353.2**	**188**	**150**	**20**	**2**	**5.7**	**3**
		105		40			
Acetylfentanyl	323	188	135	20	2	6.1	3
		105		**36**			
beta-Hydroxythiofentanyl	**359.2**	**341.2**	**135**	**12**	**2**	**6.4**	**3**
		192.1		20			
Ocfentanil	**371.2**	**188.1**	**135**	**20**	**7**	**6.5**	**3**
		105.1		40			
alpha-Methylacetylfentanyl	337.2	202.1	125	20	2	6.8	3
		91.1		**48**			
Despropionylfentanyl	281.2	188	102	18	2	6.8	3
		105		**30**			
Tetrahydrofuranfentanyl	379.2	**188**	**132**	**22**	**2**	**8.1**	**3**
		105		46			
Acrylfentanyl	335.2	188	134	22	2	8.5	3
		105		**38**			
para-Fluoroacrylfentanyl	353.2	188.1	135	20	7	8.8	3
		105.1		**40**			
Fentanyl-D_5	342.2	188.2	140	25	2	8.9	3
		105.1		50			

ortho-Fluoroacrylfentanyl	353.2	188.1	130	20	7	9	3
		105.1		**44**			
U-47700	**329.1**	**283.9**	**100**	**14**	**2**	**9.2**	**3**
		172.8		34			
Fentanyl	337.2	188.2	140	25	2	9.3	3
		105.1		**45**			
Para/meta-Fluorofentanyl	355.2	188.1	155	24	2	9.6	3
		105.1		**44**			
alpha-Methylfentanyl	351.2	202.2	115	20	2	9.9	3
		91.1		**50**			
Furanylfentanyl	375.2	**188**	**136**	**26**	**2**	**9.9**	**3**
		105		42			
ortho-Fluorofentanyl	355.2	188.1	115	24	2	10.3	3
		105.1		**44**			
Cyclopropylfentanyl	349.2	188.1	120	24	2	10.7	3
		105.1		**50**			
U-49900	**357.2**	**284.1**	**110**	**16**	**7**	**10.9**	**3**
		172.9		36			
Carfentanil	**395.2**	**335**	**112**	**18**	**2**	**11.5**	**3**
		113		34			
cis-3-Methylfentanyl	351.3	202	142	22	2	11.7	3
		105		**46**			
trans-3-Methylfentanyl	351.3	202	136	22	2	11.7	3
		105		**42**			
Butyrylfentanyl	351.3	188	138	22	2	12.4	3
		105		**42**			
Para/meta-Fluoroisobutyrylfentanyl	**369.2**	**188**	**140**	**22**	**2**	**12.4**	**3**
		105		46			
Phenylfentanyl	385.2	188	150	20	2	12.6	3
		105		**44**			
3-Fluorofentanyl	355.2	186.1	120	20	2	12.7	3
		206.1		**24**			
Benzodioxolefentanyl	429.2	188	140	24	2	12.7	3
		149		**28**			
Para/meta-Fluorobutyrylfentanyl	**369.2**	**188.1**	**135**	**24**	**2**	**12.7**	**3**
		105.1		48			
ortho-Fluoroisobutyrylfentanyl	369.2	188.1	115	24	7	13	3
		105.1		**44**			
ortho-Fluorobutyrylfentanyl	369.2	188.1	145	24	7	13.4	3
		105.1		**44**			
4-Methoxybutyrylfentanyl	381.3	188.1	165	24	2	13.5	3
		105.1		**50**			

Valerylfentanyl	365.3	188	146	22	2	14.6	3
		105		**46**			

28.5.8.3 Data Analysis Parameters (Note: Retention times listed are for elution order reference and may vary by system.)

Target	Approximate Retention Time	RRT Acceptance (%)
Methoxyacetylfentanyl	5.7	1
Acetylfentanyl	6.1	1
beta-Hydroxythiofentanyl	6.4	0.8
Ocfentanil	6.5	0.8
alpha-Methylacetylfentanyl	6.8	1
Despropionylfentanyl	6.8	2
Tetrahydrofuranfentanyl	8.1	0.5
Acrylfentanyl	8.5	0.5
para-Fluoroacrylfentanyl	8.8	0.5
ortho-Fluoroacrylfentanyl	9	0.5
U-47700	9.2	0.8
Fentanyl	9.3	0.5
para/meta-Fluorofentanyl	9.6	0.5
alpha-Methylfentanyl	9.9	0.5
Furanylfentanyl	9.9	1
ortho-Fluorofentanyl	10.3	0.8
Cyclopropylfentanyl	10.7	0.8
U-49900	10.9	1.5
Carfentanil	11.5	1
trans-3-Methylfentanyl	11.7	1
cis-3-Methylfentanyl	11.7	1
Butyrylfentanyl	12.4	1
para/meta-Fluoroisobutyrylfentanyl	12.4	1
Phenylfentanyl	12.6	1.5
3-Fluorofentanyl	12.7	1.5
Benzodioxolefentanyl	12.7	1.5
para/meta-Fluorobutyrylfentanyl	12.7	1
ortho-Fluoroisobutyrylfentanyl	13	1
ortho-Fluorobutyrylfentanyl	13.4	1
4-Methoxybutyrylfentanyl	13.5	1.5
Valerylfentanyl	14.6	2

28.6 Procedure

Note: Extracts from this method may be analyzed for OpiCoc targets and fentanyl derivatives individually or simultaneously.

28.6.1 Note: Urine specimens may be hydrolyzed to remove glucuronide conjugates prior to extraction using one of the following hydrolysis procedures. A glucuronide positive control should be used to ensure the hydrolysis was effective.

28.6.1.1 Enzyme hydrolysis**:** Add 5000 Fishman units of β-glucuronidase to each mL of urine. Perform hydrolysis as recommended by the supplier based on the source of β-glucoronidase (e.g., 5000 F units/mL Patella vulgata in 100 mM acetate buffer (pH 5.0) hydrolyzed at 65°C for 3 hours).

28.6.2 Label appropriate clean screw cap tubes accordingly, negative, calibrators, control(s) and case sample IDs

28.6.3 Prepare calibrators and controls. To eliminate a solvent effect, calibrators and controls shall be dried under nitrogen prior to the addition of blank blood. (Volume (µL) to deliver into the appropriately labeled screw top tubes)

Cal 1-A 1.0/10.0 mg/L (µL)	Cal 1-B 0.1/1.0 mg/L (µL)	Cal 2-A 10.0 mg/L (uL)	Cal 2-B 1.0 mg/L (µL)	Final Concentration Calibrator 1 Targets (mg/L)	Final Concentration Calibrator 2 Targets (mg/L)
-	10	-	10	0.001/0.01	0.01
-	20	-	20	0.002/0.02	0.02
-	50	-	50	0.005/0.05	0.05
-	100	-	100	0.01/0.1	0.1
25	-	20	-	0.025/0.25	0.2
75	-	40	-	0.075/0.75	0.4
100	-	80	-	0.1/1.0	0.8
200	-	100	-	0.2/2.0	1

Calibrator 1 Targets:

1.0 mg/L and 0.1 mg/L Oxymorphone, Hydromorphone, 6-Monoacetylmorphine, Acetyl Fentanyl, Fentanyl

10.0 mg/L and 1.0 mg/L Benzoylecgonine, Meperidine, Tramadol, Methadone

Calibrator 2 Targets:

10.0 mg/L and 1.0 mg/L Morphine, Codeine, Oxycodone, Hydrocodone, Cocaethylene, Cocaine

Qualitative Fentanyl Analog Control Preparation

A low threshold control, high carryover control, and negative control shall be assessed with each fentanyl analog quantitative analysis

Control Level	Qualitative Mix A Volume of 0.5/1.0 mg/L (µL)	Qualitative Mix B Volume of 0.005/0.01 mg/L (µL)	Final Concentration Qualitative Control (mg/L)
Low Threshold Control	-	50	0.00025/0.0005
High Carryover Control	100	-	0.05/0.1

28.6.4 Pipet 1.0 mL of blank blood, calibrators, controls and case sample bloods, fluids or tissue homogenates in appropriately labeled tubes

28.6.5 Add 20 µL 1.0/2.5 mg/L working internal standard solution to each tube

28.6.6 Vortex briefly

28.6.7 Add 2.0 mL of acetonitrile to each tube and vortex for 15-30 seconds. Ensure that the blood in the bottom of the tube mixes thoroughly with the acetonitrile

28.6.8 Centrifuge at approximately 2800 rpm for 15 minutes to achieve separation

28.6.9 Freeze samples for approximately 30 minutes at approximately -20 ºC (Note: upon removal from freezer there should be three distinct layers)

28.6.10 Transfer topmost layer into conical bottom tubes and evaporate to dryness at approximately 50-60 °C under nitrogen

28.6.11 Reconstitute in 200 µL 0.01% formic acid and 6 mM ammonium formate in water. (Note: Centrifugation may be necessary at this step)

28.6.12 Transfer to autosampler vials

28.7 Quality Control and Reporting

28.7.1 OpiCoc Quality Control and Reporting

28.7.1.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte.

28.7.1.2 The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

28.7.1.3 6-monoacetylmorphine may only be reported if morphine is confirmed in the same sample or any other biological specimen from the same individual (e.g., 6-monoacetylmorphine positive in urine and morphine positive in blood). Depending on case history and circumstances, 6-monoacetylmorphine may be reported as positive without morphine present after supervisor approval.

28.7.1.4 When a target concentration is above the ULOQ, 1.0 mL of case sample shall be diluted with no more than 19.0 mL of blank blood for a total dilution volume of 20.0 mL. Alternatively, 0.05 mL of case sample may be used for a dilution of 1/20. If a dilution greater than 1/10 is required, a positive displacement pipette shall be used for the delivery of case sample. If less 0.05 mL of sample is used for analysis, only qualitative results may be reported.

28.7.1.5 The calibration model for all targets is weighted (1/x) quadratic with the exception of morphine, tramadol, and cocaine which are weighted (1/x) linear. Samples with a concentration greater than the second highest calibrator concentration for a target with a quadratic fit must be repeated if the high positive control is outside of acceptable limits.

28.7.1.6 Extracted samples are stable for seven days after reconstitution.

28.7.1.7 Calibrator and control solutions may be made in larger than 10 mL volumes, if necessary. The larger volume calibrator solutions need to be made in calibrated glassware.

28.7.2 Fentanyl Derivatives Quality Control and Reporting

28.7.2.1 The presence of fentanyl derivatives may only be reported if the instrumental response ratio is equal to or greater than the low threshold control response ratio and all other qualitative identification criterion are met. If the instrumental response of a case sample is greater than the high carryover control, an evaluation of carryover shall be performed.

28.7.2.2 Any targets that are outside of the relative retention time (RRT) acceptance range shall not be reported.

28.7.2.3 Extracted samples are stable for seven days after reconstitution.

28.7.2.4 The high and low controls for the fentanyl derivatives shall be averaged for the RT and the transition ratios.

28.8 References

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29 Buprenorphine, Norbuprenorphine and Naloxone Quantitation and Confirmation by LCMSMS

29.1 Summary

Buprenorphine, norbuprenorphine and naloxone are extracted from biological samples using solid phase extraction (SPE) and confirmed or quantified with LCMSMS. These drugs may be analyzed simultaneously or individually as needed.

Naloxone may not be run with every batch. This can be run by customer request or at the discretion of the toxicology staff assigning testing.

29.2 Specimen Requirements

2 mL of blood or fluid. Note: This method is not suitable for tissue homogenate analysis.

29.3 Reagents and Standards

- Drugs and associated internal standards

Target	Internal Standards
Buprenorphine	Buprenorphine-D_4
Norbuprenorphine	Norbuprenorphine- D_3
Naloxone*	Naloxone- D_5

*May not be run with every batch

- Acetonitrile, HPLC grade
- Dichloromethane, ACS grade or better
- Methanol, ACS grade or better
- 2-Propanol, ACS grade or better
- HPLC grade water
- Distilled water (dH2O)
- Formic acid, Fisher Optima LC/MS
- Glacial Acetic Acid
- Potassium Phosphate
- Ammonium Hydroxide

29.4 Solutions, Internal Standard, Calibrators and Controls

29.4.1 1 M Acetic acid: Dilute 5.75 mL of glacial acetic acid with dH_2O to 100 mL. Store at room temperature for up to two years.

29.4.2 0.1 M Phosphate buffer, pH 6.0: Weigh 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of dH_2O. Adjust the pH of the solution to 6.0 by the addition of 5.0 M potassium hydroxide while stirring. QS to volume using dH_2O. Store at room temperature for up to 2 years.

29.4.3 Elution solvent (dichloromethane, 2-propanol, ammonium hydroxide, 77/20/3): Combine 20 mL of 2-propanol, and 3.0 mL of ammonium hydroxide then add 77 mL of dichloromethane. pH should be between 11-12. Make fresh for each day of use.

29.4.4 Mobile phase A (HPLC grade water with 0.1% formic acid): Add 1.0 mL of formic acid to approximately 0.5 L of HPLC grade water and qs to 1.0 L with HPLC grade water.

29.4.5 Mobile phase B (HPLC grade acetronitrile with 0.1% formic acid): Add 1.0 mL of formic acid to approximately 0.5 L of HPLC grade acetonitrile and qs to 1.0 L with HPLC grade acetonitrile.

29.4.6 Preparation of working internal standard (IS) solution (0.10 mg/L)

29.4.6.1 Dilute 10 µL of each internal standard (buprenorphine- D_4, norbuprenorphine- D_3, naloxone- D_5) stock solution (100 µg/mL) in a 10 mL volumetric flask with acetonitrile. Store in freezer.

29.4.7 Preparation of calibrators

29.4.7.1 Working calibrator solution A (CAL A) (2.0 mg/L): Pipet 20 µL of each 1.0 mg/mL standard stock solution (buprenorphine, norbuprenorphine, and naloxone) in a calibrated 10 mL volumetric flask with acetonitrile. Store in freezer.

29.4.7.2 Working calibrator solution B (CAL B) (0.2 mg/L): Pipet 1000 µL of CAL A (2.0 mg/L) in a calibrated 10 mL volumetric flask with acetonitrile. Store in freezer.

29.4.7.3 Working calibrator solution C (CAL C) (0.02 mg/L): Pipet 1000 µL of CAL B (0.2 mg/L) in a calibrated 10 mL volumetric flask with acetonitrile. Store in freezer.

29.4.7.4 To prepare the calibration curve, pipette the following volumes of CAL A, B, or C working calibrator solutions into appropriately labeled 16 x 125 mm screw cap test tubes. DO NOT dry down calibrators with nitrogen or heat. Add 2 mL blank blood to obtain the final concentrations listed below. Mix by vortex.

Calibrator Concentration (mg/L)	Calibrator Working Solution	Volume of Working Solution (µL)
0.020	CAL A – 2.0 mg/L	20
0.015	CAL A – 2.0 mg/L	15
0.010	CAL B – 0.2 mg/L	100
0.0075	CAL B – 0.2 mg/L	75
0.0050	CAL B – 0.2 mg/L	50
0.0025	CAL B – 0.2 mg/L	25
0.0010	CAL C – 0.02 mg/L	100
0.0005	CAL C – 0.02 mg/L	50
0.00025 LOD	CAL C– 0.02 mg/L	25

29.4.7.5 Preparation of controls

29.4.7.5.1 Working control solution A (CTL A) (2.0 mg/L): Pipet 20 µL of each 1.0 mg/mL standard stock solution (buprenorphine, norbuprenorphine, and naloxone) in a 10 mL volumetric flask with acetonitrile. Store in freezer.

29.4.7.5.2 Working control solution B (CTL B) (0.2 mg/L): Pipet 1000 µL of CTL A (2.0 mg/L) in a 10 mL volumetric flask with acetonitrile. Store in freezer.

29.4.7.5.3 Working control solution C (CTL C) (0.040 mg/L): Pipet 2000 µL of CTL B (0.2 mg/L) in a 10 mL volumetric flask with acetonitrile. Store in freezer.

29.4.7.5.4 To prepare positive controls, the following are provided as examples of concentrations and instructions. Pipette the following volumes of CTL A and B working control solutions into appropriately labeled 16 x 125 mm screw cap test tubes. Do not dry down controls with nitrogen or heat. Add 2 mL blank blood to obtain the final concentrations listed below. Mix by vortex.

Positive Control Concentration (mg/L)	Control Solution	Working Solution Volume (µL)
0.0020	CTL C (40 µg/L)	100
0.0080	CTL B (200 µg/L)	80
0.017	CTL A (2000 µg/L)	17

29.4.7.5.5 Negative control: Blood bank blood or equivalent determined not to contain buprenorphine, norbuprenorphine, or naloxone.

29.5 Apparatus

29.5.1 Test tubes, round bottom, borosilicate glass with Teflon caps

29.5.2 Test tubes, conical bottom

29.5.3 Test tube Rotator

29.5.4 Centrifuge capable of 3500 rpm

29.5.5 Solid phase extraction columns (SPE) UCT ZSDAU020

29.5.6 United Chemical Technologies 48 place positive pressure manifold or suitable substitute

29.5.7 Evaporator/concentrator

29.5.8 Vortex mixer

29.5.9 GC autosampler vials with inserts

29.5.10 Typical LCMSMS parameters. The following instrument conditions may be modified to adjust or improve separation and sensitivity.

- LC Parameters
- Column: Poroshell 120 EC-C18, 2.1 x 75 mm, 2.7 µmM particle size
- Column Thermostat: 50°C
- Solvent A: H_2O with 0.1% formic acid
- Solvent B: Acetonitrile with 0.1% formic acid
- Initial Flow Rate: 0.50 mL/min
- Injection vol.: 10 µL with 30 second needle wash
- Stop time: 5.50 min
- Post time: 2.00 min
- Gradient:

Time (minutes)	Solvent A	Solvent B
0.00	98.0	2.0
1.91	70.0	30.0
4.20	60	40
4.21	5	95

29.5.11 Typical MS-MS parameters. The following instrument conditions may be modified to adjust or improve sensitivity. Optimizer should be used for each compound to set the fragmentor, collision energy, and cell accelerator voltages.

- MSD Parameters:Ionization: ESI
  - Polarity: positive
  - Gas temp: 350°C
  - Drying Gas: 10.0 L/min
  - Nebulizer press: 40 psi
  - Capillary: 4000 V

- Time Segments (TS):

  - TS1: 0-2.7 minutes (To Waste)
  - TS2: 2.7-3.1 minutes (Naloxone- D_5, Naloxone)
  - TS3: 3.1-3.5 minutes (Norbuprenorphine- D_3, Norbuprenorphine)
  - TS4: 3.5-4.2 minutes (Buprenorphine- D_4, Buprenorphine)
  - TS5: 4.2-5.5 minutes (To Waste)

Analytes and IS	Transitions (m/z) Q1	Q3	Dwell (ms)	Frag (V)	CE (V)
Naloxone	328.2	**310.2**	85	160	15
		212.1	85	160	39
Naloxone- D_5	333.2	**273.2**	85	160	27
		258.2	85	160	27
Norbuprenorphine	414.3	**396.3**	60	200	29
		340.3	60	200	33
Norbuprenorphine- D_3	417.3	**101.1**	60	179	39
		83.2	60	179	55
Buprenorphine	468.3	**414.2**	65	190	35
		396.2	65	190	39
Buprenorphine- D_4	472.3	**400.2**	65	198	39
		101.1	65	198	43

Bold transition ions are used for quantification. Frag – Fragment Voltage; CE - collision energy

29.5.12 Calibration Model. Buprenorphine and naloxone calibration are linear weighted (1/x). Norbuprenorphine calibration is quadratic weighted (1/x).

29.6 Procedure

29.6.1 Label appropriate clean screw cap tubes accordingly, negative, calibrators, control(s), and case sample IDs.

29.6.2 Prepare calibrators and controls. (Note: DO NOT dry down calibrators and controls).

29.6.3 Pipet 2.0 mL of blank blood, calibrators, controls (if not already completed), and case sample bloods, or fluids into appropriately labeled tubes.

29.6.4 Add 25 µL of IS to each tube.

29.6.5 Add 2.0 mL of phosphate buffer.

29.6.6 Cap and vortex briefly to mix, then rotate tubes for 10 min.

29.6.7 Centrifuge at approximately 3500 RPM for 10 min.

29.6.8 Solid phase extraction (unless otherwise instructed column flow rate is approximately 1 mL/min with pressurized air or nitrogen).

- Wash column with 2.0 mL of methanol.
- Wash column with 2.0 mL of dH_2O.
- Wash column with 2.0 mL of phosphate buffer.
- Pour prepared sample on column. Aspirate slowly so sample takes at least two minutes to pass through the column.
- Wash column with 3.0 mL of dH_2O.
- Wash column with 3.0 mL of acetic acid.
- Wash column with 3.0 mL of methanol.

- Dry column for approximately 10 min at maximum pressure.
- Elute column with 3.0 mL of elution solvent by gravity flow into a screw-cap conical centrifuge tube.

29.6.9 Evaporate eluate to dryness at a setting of 40°C under nitrogen.

29.6.10 Reconstitute residue with 50 µL of 75% mobile phase A mixed with 25% mobile phase B.

29.6.11 Briefly vortex and pulse spin to pull extract to the bottom of the centrifuge tube.

29.6.12 Transfer to autosampler vials fitted with glass inserts.

29.7 Quality Control and Reporting

29.7.1 The LOQ for this procedure is defined as the lowest acceptable calibrator concentration used in the calibration curve for each analyte.

29.7.2 The ULOQ for this procedure is defined as the highest acceptable calibrator concentration used in the calibration curve for each analyte.

29.7.3 Due to the quadratic nature of the norbuprenorphine calibration curve, at least three controls, at low medium and high concentrations, must be run across the concentration range in every batch. If the 20 µg/L calibrator is included in the calibration curve, the 17 µg/L control or a control within 15 – 20 µg/L must be included.

29.7.4 All compounds are stable up to seven days with the exception of naloxone which is considered stable for three days.

29.7.5 See Toxicology Quality Guidelines

Note: High concentrations of amphetamine (~2.0 mg/L) and some post-mortem sample components may cause reduced internal standard signals for buprenorphine-D_4, norbuprenorphine-D_3, and naloxone-D_5. The examiner should evaluate these on a case-by-case basis and report as present at the examiner's discretion.

29.8 References

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30 Novel Psychoactive Substances Qualitative Screen and Confirmation using LCMSMS

30.1 Summary

Novel psychoactive substances are extracted from biological samples using a solid phase extraction. An aliquot of the extract is qualitatively analyzed by LCMSMS. Targets may be analyzed by panel, different combinations, or individually as needed.

Note: Novel psychoactive substances can be abbreviated as NPS. Formal names and synonyms may be included in Appendix B for the target compounds however the common names listed below will be used for reporting.

30.2 Specimen Requirements

0.5 mL of blood or fluid. Note: This method is not suitable for tissue homogenates.

30.3 Reagents and Standards

- Drug targets and associated internal standards

Panel	Target Compounds	Internal Standards
Research Chemicals	Methiopropamine	Pentylone-D_3
	3-Fluorophenmetrazine	Pentylone-D_3
	4-APDB	Pentylone-D_3
	5-APDB	Pentylone-D_3
	6-APDB	Pentylone-D_3
	Dibutylone (bk-DMBDB)	Pentylone-D_3
	4/5/6-MAPB	Pentylone-D_3
	Pentylone/N,N-Dimethylpentylone	Pentylone-D_3
	N-Ethylpentylone/N,N-Diethylpentylone	Pentylone-D_3
	5-DBFPV	Pentylone-D_3
	4-Chloro-alpha-PVP	Pentylone-D_3
	PV8	25I-NBOME-D_3
Miscellaneous	Mitragynine	Pentylone-D_3
	Methoxyphenidine	Pentylone-D_3
	Tenocyclidine (TCP)	Pentylone-D_3
	3-Methoxy-PCP	Pentylone-D_3
	Clonazolam	25I-NBOMe-D_3
Hallucinogens	25H-NBOMe	25I-NBOMe-D_3
	25C-NBOMe	25I-NBOMe-D_3
	25I-NBOH	25I-NBOMe-D_3
	25B-NBOMe	25I-NBOMe-D_3
	25I-NBF	25I-NBOMe-D_3
	25I-NBMD	25I-NBOMe-D_3
	25I-NBOMe	25I-NBOMe-D_3
Cannabimimetic Agents	5F-AB-PINACA	25I-NBOMe-D_3
	AB-FUBINACA	25I-NBOMe-D_3
	ADB-FUBICA	25I-NBOMe-D_3
	AB-PINACA	25I-NBOMe-D_3

	3F-AMB	25I-NBOMe-D_3
Cannabimimetic Agents	4F/5F-AMB	25I-NBOMe-D_3
	4F-ADB	25I-NBOMe-D_3
	5F-PB-22	25I-NBOMe-D_3
	AMB-FUBINACA	25I-NBOMe-D_3
	SDB-006	25I-NBOMe-D_3
	FUB-MDMB (MDMB-FUBINACA)	25I-NBOMe-D_3
	MAB-CHMINACA (ADB-CHMINACA)	25I-NBOMe-D_3
	PB-22	25I-NBOMe-D_3
	MMB-CHMICA	25I-NBOMe-D_3

- Potassium phosphate buffer solution concentrate (1 M, pH 6.0, e.g., Fisher)
- Potassium hydroxide
- Potassium phosphate
- Methanol, HPLC grade or higher
- Acetate buffer
- Methylene chloride
- 2-Propanol
- Ammonium hydroxide, Optima grade
- Hydrochloric acid, Optima grade
- Formic acid, eluent additive ~98%
- Type I or LCMS grade water
- Sodium acetate trihydrate
- Glacial acetic acid

30.4 Solutions, Internal Standard, Calibrators and Controls

30.4.1 0.1 M Potassium Phosphate Buffer, pH 6.0: Weigh 13.61 g of KH_2PO_4 and transfer into a 1 L volumetric flask containing approximately 800 mL of deionized water. Adjust the pH of the above solution to 6.0 by the addition of 5 M potassium hydroxide while stirring and qs to volume with deionized water. Solution may also be purchased as a 10X concentrate that must be diluted prior to use (e.g., Fisher). Dilute one volume of potassium phosphate buffer solution concentrate with nine volumes of deionized water. Store at room temperature for up to two years.

30.4.2 0.1 M Acetate Buffer: Weigh 2.93 g sodium acetate trihydrate into a 500 mL volumetric flask. Add approximately 400 mL of deionized water to dissolve sodium acetate. Add 1.62 mL glacial acetic acid. Adjust pH to 4.5 with 1 M acetic acid and qs to volume with deionized water. Store at room temperature for up to two years.

30.4.3 Dichloromethane/isopropanol/ammonium hydroxide (78:20:2): Mix 78 mL dichloromethane with 20 mL isopropanol. Mix well. In hood, add 2 mL ammonium hydroxide. Mix gently. Prepare solution fresh daily.

30.4.4 Mobile Phase A (H_2O with 0.1% formic acid): Add 1.0 mL of formic acid to 1.0 L of Type I or LCMS grade water.

30.4.5 Mobile Phase B (Methanol with 0.1% formic acid): Add 1.0 mL of formic acid to 1.0 L of methanol.

30.4.6 Reconstitution Solvent (98:2 water:methanol): Add 2.0 mL of methanol to 98.0 mL dH_2O. Different final volumes may be prepared that maintain the 98:2 volume ratio.

30.4.7 0.2% Hydrochloric acid in 2-propanol: Add 1 mL of concentrated HCl (12N) into 500 mL of 2-propanol. Store at room temperature for up to one month.

30.4.8 Working Internal Standard (1.0 mg/L): Pipette 10.0 µL of the 1.0 mg/mL or 100 µL of 0.1 mg/mL stock solution into a 10.0 mL volumetric flask and qs to volume with acetonitrile.

30.4.9 Controls

30.4.9.1 Negative control: blank blood or equivalent determined not to contain target compounds.

30.4.9.2 High Threshold Working Standard (10.0 mg/L): Pipette 100 µL of 1.0 mg/mL or 1000 µL of 0.1 mg/mL stock solution into a 10.0 mL volumetric flask and qs to volume with acetonitrile. This may be made into two different standards due to volume limitations.

30.4.9.3 Low Threshold Working Standard A (1.0/5.0/10.0 mg/L): Pipette the following volumes for each compound given a 1.0 mg/mL stock solution into a 10.0 mL volumetric flask and qs to volume with acetonitrile.

Target Compound	Volume of 1.0 mg/mL Stock Solution (µL)
Methiopropamine	10
Dibutylone	10
6-MAPB	10
5-DBFPV	10
25H-NBOMe	10
4F-AMB	10
3F-AMB	10
3-Fluorophenmetrazine	50
4-APDB	50
5-APDB	50
Pentylone	50
N-Ethylpentylone	50
Tenocyclidine	50
4-Chloro-alpha-PVP	50
3-Methoxy-PCP	50
Mitragynine	50
Methoxyphenidine	50
PV8	50
25C-NBOMe	50
Clonazolam	50
25I-NBOMe	50
5F-AB-PINACA	50
AB-FUBINACA	50
ADB-FUBICA	50
AB-PINACA	50
AMB-FUBINACA	50
4F-ADB	50
SDB-006	50
6-APDB	100
25I-NBOH	100
25B-NBOMe	100
25I-NBF	100
FUB-MDMB	100
PB-22	100
MMB-CHMICA	100
25I-NBMD	100
5F-PB-22	100
MAB-CHMINACA	100

30.4.9.4 Low Threshold Working Standard B (0.01/0.05.0.1 mg/L): Pipette 100 µL of Low Threshold Working Standard A into a 10.0 mL volumetric flask and qs to volume with acetonitrile.

30.4.9.5 Preparation of qualitative threshold controls

Control Level	High Threshold Working Standard 10.0 mg/L (µL)	Low Threshold Working Standard B 0.01/0.05/0.1 mg/L (µL)	Final Concentration Qualitative Control (mg/L)
Low Threshold Control	-	50	0.001/0.005/0.1
High Carryover Control	50	-	1.0

30.5 Apparatus

30.5.1 Agilent Technologies LCMSMS, MassHunter software, compatible computer and printer

30.5.2 Test tubes, round bottom, screw cap tubes, borosilicate glass with Teflon caps

30.5.3 Test tubes, glass tubes, conical bottom

30.5.4 Centrifuge capable of 2,000-3,000 rpm

30.5.5 Solid phase extraction cartridges (UCT CleanScreen ZSDAU020 columns)

30.5.6 Solid phase extraction manifold

30.5.7 Vortex mixer

30.5.8 Heating block

30.5.9 Evaporator/concentrator

30.5.10 GC autosampler vials and inserts

30.5.11 Agilent Technologies LCMSMS Parameters:

30.5.11.1 LC Parameters

- Column: Agilent Technologies Poroshell 120 EC-C18, 3.0x50 mm 2.7 µm (P.N. 699975-302)
- Column Thermostat: 60ºC
- Mobile Phase A: Water with 0.1% formic acid
- Mobile Phase B: Methanol with 0.1% formic acid
- Flow Rate: 0.7 mL/min
- Injection Volume: 10 µL
- Stop Time: 16.0 minutes
- Post Time: 2.0 minutes
- Gradient:

Time (minutes)	Mobile Phase A (%)	Mobile Phase B (%)
0.00	98.0	2.0
2.00	98.0	2.0
7.00	93.0	7.0
13.00	5.0	95.0
14.00	2.0	98.0
15.00	2.0	98.0
16.00	98.0	2.0

30.5.11.2 MSMS Parameters

- Ionization: ESI
- Polarity: Positive
- Gas Temperature: 325ºC
- Nebulizer Pressure: 45 psig
- Capillary: 3500 V
- Drying Gas Flow: 12 L/min
- Transition Ions: (Note: Target MRMs for all listed compounds may not be removed from the data acquisition method without Program Manager approval due to changes in sensitivity for isomeric compounds. Target transitions are in bold font, qualifier transition is in normal font.)
    - Retention times are listed below for elution order reference and may change from instrument to instrument.
    - Target transitions are formatted in bold.

Compound Name	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)	Cell Accelerator Voltage (V)	Ret Time (min)	Delta Ret Time (min)
Methiopropamine	156.1	125.0	75	8	2	3.062	3
		97.0		**20**		3.062	
3-Fluorophenmetrazine	**196.1**	**115.0**	**105**	**32**	**2**	5.348	3
		109.0		32		5.348	
4-APDB	**178.1**	**161.1**	**75**	**4**	**2**	6.280	3
		133.0		16		6.280	
5-APDB	**178.1**	**161.1**	**65**	**4**	**2**	6.870	3
		133.0		20		6.870	
6-APDB	**178.1**	**161.1**	**75**	**4**	**2**	7.600	3
		133.0		20		7.600	
Dibutylone (bk-DMBDB)	236.1	161.1	105	20	2	8.019	3
		86.1		**24**		8.019	
4/5/6-MAPB	190.1	159.1	90	8	2	8.548	3
		131.0		**20**		8.548	
Pentylone-D_3	**239.1**	**221.2**	**100**	**12**	**2**	9.000	3
		191.1		20		9.000	
Pentylone/N,N-Dimethylpentylone	236.1	218.1	85	8	2	9.080	3
		188.1		**16**		9.080	
N-Ethylpentylone/N,N-Dietheylpentylone	250.1	202.1	115	16	2	9.100	3
		232.1		**8**		9.100	
5-DBFPV	274.2	133.0	135	28	2	9.596	3
		126.1		**24**		9.596	
Tenocyclidine (TCP)	**250.2**	**165.0**	**70**	**8**	**2**	9.724	3
		86.0		4		9.724	
4-Chloro-alpha-PVP	266.1	126.1	100	28	2	10.022	3
		125.0		**24**		10.022	
3-Methoxy-PCP	**274.2**	**121.1**	**90**	**28**	**2**	10.055	3
		86.1		8		10.055	
Mitragynine	399.2	226.1	130	24	2	10.137	3

		174.1		**32**		10.137	
Methoxyphenidine	**296.2**	**211.1**	**100**	**8**	**2**	10.215	3
		117.1		20		10.215	
25H-NBOMe	302.2	121.1	120	16	2	10.232	3
		91.1		**40**		10.232	
PV8	260.2	154.1	135	28	2	10.336	3
		91.1		**24**		10.336	
25I-NBOMe-D_3	**431.1**	**124.1**	**120**	**24**	**2**	10.500	3
		92.1		25		10.500	
25C-NBOMe	**336.1**	**121.0**	**95**	**20**	**2**	10.588	3
		91.1		54		10.588	
25I-NBOH	**414.1**	**291.0**	**130**	**20**	**2**	10.627	3
		107.0		32		10.627	
Clonazolam	**354.1**	**308.1**	**135**	**28**	**2**	10.655	3
		280.1		40		10.655	
25B-NBOMe	380.1	91.1	105	58	2	10.687	3
		121.1		**16**		10.687	
25I-NBF	**416.1**	**291.0**	**135**	**20**	**2**	10.729	3
		275.9		32		10.729	
25I-NBMD	442.1	286.1	130	16	2	10.753	3
		135.0		**28**		10.753	
25I-NBOMe	**428.1**	**121.1**	**120**	**20**	**2**	10.855	3
		91.1		58		10.855	
5F-AB-PINACA	**349.2**	**304.2**	**70**	**12**	**2**	11.464	3
		233.1		20		11.464	
AB-FUBINACA	369.2	253.1	95	24	2	11.666	3
		109.1		**56**		11.666	
ADB-FUBICA	**382.2**	**252.1**	**85**	**16**	**2**	11.869	3
		109.0		48		11.869	
AB-PINACA	331.2	286.2	70	12	2	12.067	3
		215.0		**24**		12.067	
4F/5F-AMB	**364.2**	**233.1**	**115**	**20**	**2**	12.090	3
		145.0		50		12.090	
3F-AMB	**364.2**	**233.1**	**115**	**24**	**2**	12.090	3
		145.0		44		12.090	
4F-ADB	378.2	318.2	130	12	2	12.096	3
		233.1		**24**		12.096	
5F-PB-22	**377.2**	**232.1**	**80**	**12**	**2**	12.097	3
		144.0		40		12.097	
AMB-FUBINACA	384.2	253.0	125	20	2	12.222	3
		109.0		**50**		12.222	
SDB-006	321.2	214.1	145	20	2	12.339	3

		91.1		**48**		12.339	
FUB-MDMB (MDMB-FUBINACA)	398.2	253.1	105	24	2	12.458	3
		109.0		**48**		12.458	
MAB-CHMINACA (ADB-CHMINACA)	**371.2**	**241.1**	**110**	**24**	**2**	12.588	3
		145.0		44		12.588	
PB-22	**359.2**	**214.1**	**90**	**8**	**2**	12.599	3
		144.0		40		12.599	
MMB-CHMICA	**371.2**	**240.1**	**115**	**12**	**2**	12.647	3
		144.0		40		12.647	

30.5.12 Data Analysis Parameters (Note: Relative Retention Time acceptance criteria established for isomeric compounds only.)

Relative Retention Time Acceptance Criterion	
Target	Relative Retention Time Criteria (%)
4-APDB	1.5
5-APDB	1.5
6-APDB	1.5
4/5/6-MAPB	0.5
Pentylone/N,N-Dimethylpentylone	0.3
Dibutylone	1.5
N-Ethylpentylone/N,N-Diethylpentylone	0.3
3-Methoxy-PCP	0.3
PB-22	0.3
4F/5F-AMB	0.3
3F-AMB	0.3
4F-ADB	0.3

30.6 Procedure

30.6.1 Label appropriate clean screw cap tubes accordingly, negative, threshold controls and case sample IDs.

30.6.2 Prepare controls. To eliminate solvent effect, controls shall be dried down under nitrogen prior to the addition of blank blood.

30.6.3 Pipette 500 µL of blank blood, controls, and case sample blood/fluids in appropriately labeled tubes.

30.6.4 Add 10 µL of 1.0 mg/L internal standard solution to each tube.

30.6.5 Vortex briefly.

30.6.6 Add 1.0 mL of 0.1 M phosphate buffer (pH 6) to each tube and vortex.

30.6.7 Centrifuge at approximately 2800 rpm for 15 minutes to achieve separation.

30.6.8 Solid phase extraction. Place SPE cartridges in the extraction manifold. Throughout the SPE procedure, it is important not to permit the SPE sorbent bed to dry, unless specified. If necessary, add additional solvent/buffer to re-wet.

30.6.8.1 Add 1.0 mL methanol to each column and aspirate.

30.6.8.2 Add 1.0 mL 0.1 M phosphate buffer (pH 6) to each column and aspirate.

30.6.8.3 Without delay, pour specimens into appropriate SPE columns. Elute from cartridges with approximately 1-2 mL/minute flow.

30.6.8.4 Add 1.0 mL dH_2O to each column and aspirate.

30.6.8.5 Add 1.0 mL acetate buffer (pH 4.5) to each column and aspirate.

30.6.8.6 Wipe the SPE column tips with Kimwipes®. Place labeled conical test tubes in the manifold test tube rack. Be sure SPE column tips are in the designated conical tube.

30.6.8.7 Elute with 1.0 mL methanol. Dry the columns at > 10 inches of Hg for at least 2 minutes.

30.6.8.8 Elute with 3.0 mL of freshly prepared dichloromethane/isopropanol/ammonium hydroxide solution to each column. Collect eluate by gravity drain (no vacuum).

30.6.9 Add 40 µL of 0.2% HCl in isopropanol.

30.6.10 Evaporate samples to dryness at approximately 50ºC under nitrogen.

30.6.11 Reconstitute in 50 µL reconstitution solvent.

30.6.12 Transfer to autosampler vials.

30.7 Quality Control and Reporting

30.7.1 The presence of novel psychoactive substances may only be reported if the instrumental response ratio is equal to or greater than the low threshold control response ratio and all other qualitative identification criterion are met with the exception of the compounds identified below. If the response ratio is less than the low threshold control, the compounds delineated in the below table can be reported upon toxicologist review if all other qualitative identification criterion are met. If the instrumental response of a case sample is greater than the high carryover control, an evaluation of carryover shall be performed.

Reviewable Compounds
5F-AB-PINACA
AB-FUBINACA
ADB-FUBICA
AB-PINACA
5F-PB-22
4F/5F-AMB
AMB-FUBINACA
3F-AMB
4F-ADB
SDB-006
FUB-MDMB
MAB-CHMINACA
PB-22
MMB-CHMICA

30.7.2 Any targets that are outside of the relative retention time (RRT) acceptance range shall not be reported.

30.7.3 The following table represents extracted sample stability

Target Compound	Stability (Days)
Methiopropamine	7
3-Fluorophenmetrazine	7
4-APDB	7

5-APDB	7
Dibutylone (bk-DMBDB)	7
6-APDB	7
4/5/6-MAPB	7
Pentylone/N,N-Dimethylpentylone	7
N-Ethylpentylone/N,N-Diethylpentylone	7
5-DBFPV	7
Tenocyclidine (TCP)	7
4-Chloro-alpha-PVP	7
3-Methoxy-PCP	7
Methoxyphenidine	7
25H-NBOMe	7
PV8	7
25B-NBOMe	7
25C-NBOMe	7
25I-NBOH	7
25I-NBMD	7
Clonazolam	7
25I-NBF	7
25I-NBOMe	7
AMB-FUBINACA	7
4F-ADB	7
MAB-CHMINACA (ADB-CHMINACA)	3
Mitragynine	2
ADB-FUBICA	2
AB-PINACA	2
4F/5F-AMB	2
3F-AMB	2
5F-AB-PINACA	1
AB-FUBINACA	1
5F-PB-22	1
SDB-006	1
FUB-MDMB (MDMB-FUBINACA)	1
PB-22	1
MMB-CHMICA	1

30.7.4 As this method can be used for screening, it shall be reported with the screened list on the Certificate of Analysis. The language shall be in the following format:

Novel psychoactive substances – (panel)
Novel psychoactive substances – (panel, panel, panel)
Novel psychoactive substances – all panels

The “(panel)” or “(panel, panel, panel)” shall be replaced by the panel listed above.

30.8 References

30.8.1 L. Moses and R. Wagner, Qualitative Analysis of Novel Psychoactive Substances using LCMSMS In-house Development, 2017.

30.8.2 L. Moses and R. Wagner, Qualitative Analysis of Novel Psychoactive Substances using LCMSMS Validation, 2018.

30.8.3 Ambach, Lars *et al.*, Detection and quantification of 56 new psychoactive substances in whole blood and urine by LC-MS/MS. Bioanalysis 7(9), 1119-1136, 2015.

30.8.4 Bertol, Elisabetta *et al*., A novel screening method for 64 new psychoactive substances and 5 amphetamines in blood by LC-MS/MS and application to real cases. Journal of Pharmaceutical and Biomedical Analysis 129, 441-449, 2016.

30.8.5 Scientific Working Group for Forensic Toxicology (SWGTOX) Standard practices for method validation in forensic toxicology. *JAT* 2013, 37, 452-474.

Appendix A - Abbreviations

The following is a list of abbreviations commonly used in the Section. This list has been generated to assist in the interpretation of case files. The abbreviations are appropriate written in either lower or upper case, with and without punctuation such as periods. Empirical formulas, chemical, mathematical and shorthand abbreviations are equally acceptable and are not listed here. In all instances, abbreviations used in note taking should be readily interpretable within the context of the subject material and in conjunction with the associated Certificate of Analysis.

A.1 General Abbreviations

Abbreviations	Definitions
/nor	desmethyl metabolite
?	unidentified/unknown
A/N	acidic/neutral drugs
ABC	Alcoholic Beverage Commission
Abs	absorbance
Accd	accepted
Acids	acidic drugs
ADM	administrative
Alc	alcohols/alcohol screen
Anal	analysis
AP	Abused panel (ELISA)
Ave, Avg	average
Bases	basic drugs
BB	Blank Blood
Blk	Blank
BAC	blood alcohol content
Bld	blood
Bldy	bloody
Brn	brain
BD	Breakdown
BD prod	breakdown product
BSC	Base screen
BSQ	Base quant
C, cont	container
Cal, Calib	calibrator
c/o	Carryover
cav fl	cavity fluid
Cer	Cerilliant
Chol	Cholesterol
CHROMAT, chrom	chromatograph
cntl, ctl, cont, con, ctrl	control
Conc	concentrated/concentration
conf, conf'd	confirmed

Cpds	compounds
CSF	cerebral spinal fluid
Derive, deriv	Derivative
DAD	Diode array detector
dec fl, decomp fl	decomposition fluid
dH2O, DiH2O	deionized water
dil, diln	dilution/diluted
DNE	did not extract
DP	ELISA duid panel
DOA	Drugs of Abuse
ELISA	Enzyme Linked Immunosorbant Assay
EtOAc	ethyl acetate
Ext	extraction/extracted
Exp	Expires, expiration
EIP	extracted ion profile
F	femoral
FD	Found
FID	flame ionization detector
Form	formaldehyde
FRAGS	fragments
Fs	full scan
gst, gast	gastric
Gastric, gst, gc	gastric contents
GC	gas chromatograph/gas chromatography
GC-MS	gas chromatograph/mass spectrometer
GT	greater than
H, Ht	heart
Hb	hemoglobin
HB, Hbl	heart blood
Hex	Hexane
HbCO	carboxyhemoglobin
HBLD, Hbl, hosp	hospital blood
Homo	homogenate
HSPB	heat sealed plastic bag
ID	identification
I	Iliac
Immunal	Immunalysis
Imp	Impurities
int, integ	Integration
Int Std, ISTD, IS	internal standard
IT, ITM	item
IVC	inferior vena cava

L	left
LCMS	liquid chromatography/mass spectrometry
LCMSMS	liquid chromatography/ tandem mass spectrometry
Liq	liquid
Liv, lvr	liver
LL	liquid/liquid
LLE	liquid/liquid extraction
LLOQ	lower limit of quantitation
LOD	limit of detection
LOQ	limit of quantitation
LT	less than
Man	Manual
MC	Multicomponent
MS	mass spectrometer
MSD	mass spectrometer detector
Metab	Metabolite
Mod	moderate or moderately
N, neg	negative
NA	not analyzed
N/A	not available, not applicable
NCI	negative chemical ionization
ND	not detected or none detected
NDD	no drugs detected
Neutrals	neutral drugs
NOA	no other acidic or neutral drugs
NOB	no other alkali extractable drugs
NPD	nitrogen phosphorous detector
OpiCoc, opicoc, Opi-Coc, opi-coc	Opioid, Cocaine, Benzoylecgonine, Cocaethylene Quantitation and Confirmation by LCMSMS
O:, op, OP'D, OPN'D	opened
ORG, orig	original
PdCl2	palladium chloride
Pf	purge fluid
PGT, PRGT	present greater than
Pk	peak
Pl fl	pleural fluid
PLB	plastic bag
prep'd	prepared
PLT, PRLT	present less than
PM	Preventive maintenance, Program Manager, Post Mortem, Procedures Manual
PMP	ELISA Postmortem Panel
Pos	Positive, position (on Alcohol QC Worksheet)

Poss	Possibly
Pr	present
Prob	probably
Pur	purge
Q	quantity
QA	quality assurance
QC	quality control
QNS, QNSFA	quantity not sufficient for analysis
QNS, QNSFFA	quantity not sufficient (insufficient) for further analysis
QQQ	liquid chromatography/mass spectrometry triple quadrupole
Qs	add quantity sufficient to bring up to volume
Qual	Qualitative
Quant	Quantitation, Quantitative
R	Right, review
R:, rec'd, rcvd	received
RRT	relative retention time
Rel	Related compounds
Relinq	relinquished
Rpt, rpt, RPT	repeat
RT	retention time
Samp	Sample
SBX	sealed box
SC	Subclavian
scr, scrn	screen
SD	standard deviation
SENV	sealed envelope
Ser	Serum
SIM	selected ion monitoring
Sl	slightly
SPB	sealed plastic bag
Solv	Solvent
SPE	solid phase extraction
SPLB	sealed plastic bag
Std	standard
Subclav	Subclavian
Supraclav	supraclavian or supraclavicular
Syr	syringe
Sys	systemic/system
sys imps	systemic impurities
T, tgt	Target
T1	Tray 1
T2	Tray 2

TIC	total ion chromatogram
THIA	toluene/hexane/isoamyl alcohol
TO	Toxicology-Other
Tox, TX	Toxicology
Tr	trace
UFA, unsuit, Uns	Unsuitable for analysis
ULOL	upper limit of linearity
ULOQ	upper limit of quantitation
Unid	unidentified
UoM	Uncertainty of Measurement
UR	urine
UV	ultraviolet spectrophotometer
UV-VIS	Ultraviolet/visible spectrophotometer
V	vial
VC	vena cava
VH, vit	vitreous humor
vol, vols	volatile(s)
WB	Whole blood
WQA	Wine Quality Assurance
Wkstd	working standard
XP	ELISA extra panel
ZPLB/T	Ziploc plastic bag sealed with tape

A.2 Drug Names

Abbreviations	**Definitions**
3FF	3-fluorofentanyl
4-ANPP, desprop	Despropionyl fentanyl
4MBF	4-methoxybutyrylfentanyl
6AM, 6MAM, MAM	6-monoacetylmorphine
7-AC	7-aminoclonazepam
Ace	acetone
alp, alpraz	alprazolam
ami, amit, amitrip	amitriptyline
amobarb, amob	amobarbital
Amox	amoxepin
Amp	amphetamine
Amps	amphetamine type drugs
APAP	acetaminophen
ASA	salicylate
Atomox	atomoxetine
barb, barbs, BR	barbiturates

BE, BZG	benzoylecgonine
BE-d3	benzoylecgonine d3
Benz, benzos, BZ, benzo	benzodiazepines
benztr, benztrp	benztropine
Bromphen	brompheniramine
BUP, bupren	buprenorphine
Buprop	Bupropion
Butal	butalbital
Busp	buspirone
Caff	caffeine
Cannabs	cannabinoids
Carbam	carbamazepine
Carboxy-THC, THC-COOH	THC carboxylic acid
cariso, caris, CAR	carisoprodol
Cbd	cannabidiol
Cbn	cannabinol
cis-3-MF	cis-3-methylfentanyl
CO	carbon monoxide
CMA, Ceterz MeOH Ad	Ceterizine Methanol Adduct
CE	cocaethylene
CHLORDIAZ	chlordiazepoxide
Chlorphen	chlorpheniramine
Chlorprom	chlorpromazine
CIT, citalo, cital	citalopram
Clomip	clomipramine
Clonaz, clo	clonazepam
Cloz	Clozapine
COC	cocaine
coc metab	cocaine metabolite (benzoylecgonine)
Cod	codeine
Cot	cotinine
CYCLOBENZ, cyclobenz, cycbnz	cyclobenzaprine
Desip	Desipramine
DEX, DXM, dextro	dextromethorphan
dia, DIAZ	diazepam
DFE	difluoroethane
diltz, diltaz, diltiaz	diltiazem
DIP, DPH, DIPH, DIPHEN	diphenhydramine
DOX, doxep	doxepin
Doxyl	doxylamine
EG, ETH GLY	ethylene glycol
ETOH	ethanol

Fent, FEN	fentanyl
Fent deriv, fentalogs	Fentanyl derivatives or fentanyl analogs (both versions are synonymous)
FLUNITRAZ, flunit	flunitrazepam
FLUOX	fluoxetine
Fluraz	flurazepam
Fluvox	fluvoxamine
Gabap, Gaba	Gabapentin or gamma-aminobutyric acid
GBL	gamma butyrolactone
GHB	gamma hydroxybutyrate
Gluteth	glutethimide
Halop	haloperidol
HCD, HYC, hydrocod	hydrocodone
HYM, hydromorph	hydromorphone
HXZ	hydroxyzine
Imip	imipramine
IPA, iso, isoprop, ISOP	isopropyl alcohol or isopropanol
ketam, ket	ketamine
lamot, lamo	lamotrigine
Levam	levamisole/tetramisole
lid, lido	lidocaine
Loper	loperamide
lor, loraz	lorazepam
MC	multi-component
MDA	3,4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxymethamphetamine
MDO, Mdon, MTD, MDN	methadone
MDPV	3,4-methylenedioxypyrovalerone
Mecliz	meclizine
MeOH	methanol
MeO	Methoxy
Meper	meperidine
Mepro	meprobamate
Metax	metaxalone
meth, methamp	methamphetamine
methapyr, mp	methapyrilene
Methocarb	methocarbamol
Metoclo	metoclopramide
mFBF	meta-fluorobutyrylfentanyl
mFF	meta-fluorofentanyl
mFIBF	meta-fluoroisobutyrylfentanyl
Midaz	midazolam
MIRTAZ	mirtazapine

MJ	marijuana
mor, morph	morphine
MPZ	methadone/pcp/zolpidem
Mthphen, MPD	methylphenidate
Nalox, nalox	naloxone
Nic	nicotine
Norbup	Norbuprenorphine
Norchlor	norchlorcyclizine
nor, nordiaz	nordiazepam
Norflox	norfluoxetine
norprop, norpropx	norpropoxyphene
Nortrip	nortriptyline
n-prop	n-propanol
oFAF	ortho-fluoroacrylfentanyl
oFBF	ortho-fluorobutyrylfentanyl
oFF	ortho-fluorofentanyl
oFIBF	ortho-fluoroisobutyrylfentanyl
OLANZ	olanzapine
OP, opi	opiate(s)
Orphan	orphenadrine
OXAZ	oxazepam
oxcarbaz, oxcarb	oxcarbazepine
OXC, oxy, oxycod	oxycodone
OXM, oxymor	Oxymorphine, oxymorphone
PAROX	paroxetine
p-clamp, pclamp	p-chloroamphetamine
PCP	phencyclidine
pentobarb, pentob	pentobarbital
pFAF	para-fluoroacrylfentanyl
pFBF	para-fluorobutyrylfentanyl
pFF	para-fluorofentanyl
pFIBF	para- fluoroisobutyrylfentanyl
Phenmet	phenmetrazine
PHENOBARB, pheno, phenob	phenobarbital
Phenaz	phenazepam
Phent	phentermine
PROMETH	promethazine
PROP GLY, PG	propylene glycol
Propox	propoxyphene
Ritacid	ritalinic acid
QUET	quetiapine
secobarb, secob	secobarbital

SERT	Sertraline
Sertis	sertraline internal standard
Tapen	tapentadol
TCA, TRI	Tricyclic antidepressants
TEMAZ	temazepam
THC	tetrahydrocannabinol
THFF	tetrahydrofuranfentanyl
Triaz	triazolam
Thiorid	thioridazine
TRAM, tram	tramadol
trans-3-MF	trans-3-methylfentanyl
TRAZ	trazodone
MCPP, mCPP, CPP	trazodone metabolite - meta chlorophenylpiperazine
VA, valp, VPA	valproic acid
VENLA	venlafaxine
Verap	verapamil
Zolp	zolpidem
ZZZ	Zolpidem, zaleplon, zopiclone

A.3 Case History

Abbreviations	Definitions
Aa	automobile accident
ACI	acute coronary insufficiency
ASCVD	Arteriosclerotic cardiovascular disease
Ass	assault
BP	blood pressure
c/o	complained of
CAD	coronary artery disease
COD	cause of death
D	driver
Decomp	Decomposed
DFSA	drug-facilitated sexual assault
DIB	dead in bed
Dec	Decedent
Dep	depression
DM	diabetes mellitus (diabetic)
Dx	diagnosis
DZ	disease
Fx	fracture
GI	gastrointestinal

GSW	gunshot wound
H	homicide
h/o	history of
Hdz	heart disease
Hrnd	heart related natural death
Ht	heart
Hem	hemorrahage
HTN	hypertension
Hx	history
IH	in-house
L#	Lot number
Lac	laceration
Lipo	Lipomed
LKA	last known alive
LSA	last seen alive
MEO	medical examiner office
Mc	motorcycle
MCC	motorcycle collision
ME	medical examiner
MOD	manner of death
MVA	motor vehicle accident
MVC	motor vehicle collision
Nat	natural
NPS	Novel psychoactive substance
OD	overdose
OTJ	on the job
P, pass	passenger
PE	pulmonary embolism
Ped	pedestrian
Perf	perforated
PMP	prescription monitoring program
Rx	prescription
PTB	presumed to be
Psych	Psychiatric
r/o	rule out
s/i	suicidal ideations
s/a	suicidal attempts
S, suic	suicide
SCHIZ	Schizophrenia
SGW	shotgun wound
SD	sudden death
SDH	subdural hematoma

Sgsw	single gunshot wound
SUD	sudden unexpected death
SUID	sudden unexpected infant death
Surg	surgery/surgical
Sx	sexual
SA	sexual assault
SZ	seizure
Unresp	unresponsive
V	vehicle/vehicular
Vic	victim

A.4 DUI/DUID Evidence Codes – DUID Worksheet

Abbreviations	Definitions
CBW	Certificate of Blood Withdrawal
OA	Name order different on RFLE.
QN	Quantity insufficient for further analysis
01	Received broken vial. No analysis possible. Blood and tube discarded.
02	Container not sealed. Vial sealed.
03	Last name on Certificate of Blood Withdrawal not present.
04	Blood vial was not provided by the Department.
05	Blood was coagulated when received. No analysis was possible.
06	Spelling of last name on CBW is questionable.
07	Spelling of first name on CBW is questionable.
08	Name order on CBW is questionable.
09	First name on CBW is not legible.
10	Last name on CBW is not legible.
11	The quantity of blood received was insufficient for analysis.
12	First and last names on CBW are not legible.
13	There is no information on the CBW.
14	Spelling of first and last names on the CBW is questionable.
24	Container sealed. Vial not sealed.
27	Neither vial nor container was sealed.
30	There was no CBW submitted.
31	CBW was detached from the vial at the perforation.
33	The vial number on the vial does not match the vial number on the CBW.
41	Blood vial cracked and leaking when received.
44	Vial number is not complete.
53	CBW not fully recoverable from vial.
55	Vial cap loose. No blood available for analysis.
58	CBW not recoverable from vial.
65	The attached is a photocopy of the vial label.
66	Vial leaking.
67	No analysis performed.
68	Sample may be analyzed if resubmitted with identifying information.
69	No name on CBW.
70	CBW was not attached to the vial.

Appendix B – Testing Panel Summary

Methods:
Immunoassay (EIA)
Gas Chromatography/Mass Spectrometry (GCMS)
Liquid Chromatography (LC)
Spectrophotometry (S)
Headspace Gas Chromatography (HSGC)
Gas Chromatography (GC)
Liquid Chromatography/Tandem Mass Spectrometry (LCMSMS)

List of drugs is not all-inclusive. Additional qualitative analyses may be performed at the discretion of a toxicologist.

Toxicology Laboratory Procedure	Specimen Requirement	Screen	Qual. Testing	Quant. Testing	Method(s)	Chapter(s)
1-(1-benzo[b]thien-2-ylcyclohexyl)-piperidine (BCP)	2 mL		X		GCMS	9
1,4-Butanediol	0.2 mL		X		GCMS	15
25B-NBOMe (formal name: 4-bromo-2,5-dimethoxy-N-[(2-methoxyphenyl)methyl]-benzeneethanamine; synonyms: 2C-B-NBOMe)	0.5 mL		X		LCMSMS	30
25C-NBOMe (formal name: 2-(4-chloro-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine; Synonym: 2C-C-NBOMe)	0.5 mL		X		LCMSMS	30
25H-NBOMe (formal name: 2-(2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine; synonym: 2C-H-NBOMe)	0.5 mL		X		LCMSMS	30
25I-NBF (formal name: N-(2-fluorobenzyl)-2-(4-iodo-2,5-dimethoxyphenyl)ethanamine; synonym: 2C-I-NBF)	0.5 mL		X		LCMSMS	30
25I-NBMD (formal name: N-(benzo[d][1,3]dioxol-4-ylmethyl)-2-(4-iodo-2,5-dimethoxyphenyl)ethanamine; synonym: Cimbi-29)	0.5 mL		X		LCMSMS	30
25I-NBOH (formal name: 2-(((4-iodo-2,5-dimethoxyphenethyl)amino)methyl)phenol; synonym: 2C-I-NBOH)	0.5 mL		X		LCMSMS	30
25I-NBOMe (formal name: 4-iodo-2,5-dimethoxy-N-[(2-methoxyphenyl)methyl]-benzeneethanamine; synonym: 2C-I-NBOMe)	0.5 mL		X		LCMSMS	30
(2-methylaminopropyl)benzofuran (MAPB)	2 mL		X		GCMS	9
3F-AMB (formal name: methyl (1-(3-fluoropentyl)-1H-indazole-3-carbonyl)-L-valinate; synonym: 3-fluoro-AMP)	0.5 mL		X		LCMSMS	30
3-fluorofentanyl	1mL		X		LCMSMS	28

Toxicology Laboratory Procedure	Specimen Requirement	Screen	Qual. Testing	Quant. Testing	Method(s)	Chapter(s)
3-fluorophenmetrazine	0.5 mL		X		LCMSMS	30
3-methoxy-PCP	2 mL, 0.5mL		X		GCMS, LCMSMS	9, 30
3-methylfentanyl	2 mL		X		GCMS	17
4-APDB (formal name: 2,3-dihydro-α-methyl-4-benzofuranethanamine; synonym: 4-(2-Aminopropyl)-2,3-dihydrobenzofuran)	0.5 mL		X		LCMSMS	30
4-chloro-α-PVP (formal name: 1-(4-chlorophenyl)-2-(1-pyrrolidinyl)-1-pentanone)	0.5 mL		X		LCMSMS	30
4F-ADB (formal name: methyl (2S)-2-(1-(4-fluoropentyl)-1H-indazole-3-carboxamido)-3,3-dimethylbutanoate; synonym: 4-fluoro-MDMB-PINACA)	0.5 mL		X		LCMSMS	30
4F/5F-AMB (formal name: methyl (1-(4/5-fluoropentyl)-1H-indazole-3-carbonyl)-L-valinate; synonym: 4/5-fluoro-AMB)	0.5 mL		X		LCMSMS	30
4/5/6-MAPB (formal name: 1-(benzofuran-4/5/6-yl)-N-methylpropan-2-amine; synonym: 4/5/6-(2-Methylaminopropyl)Benzofuran)	0.5 mL		X		LCMSMS	30
4-methoxybutyrylfentanyl	1mL		X		LCMSMS	28
4-methyl-N-ethylcathinone (4-MEC)	2 mL		X		GCMS	9
5-APDB (formal name: 2,3-dihydro-α-methyl-5-benzofuranethanamine)	0.5 mL		X		LCMSMS	30
5-DBFPV (formal name: 1-(2,3-dihydrobenzofuran-5-yl)-2-(pyrrolidin-1-yl)pentan-1-one; synonym: 3-desoxy-3,4-MDPV)	0.5 mL		X		LCMSMS	30
5F-AB-PINACA (formal name: N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-(5-fluoropentyl)-1H-indazole-3-carboxamide)	0.5 mL		X		LCMSMS	30
5F-PB-22 (formal name: 1-(5-fluoropentyl)-8-quinolinyl ester-1H-indole-3-carboxylic acid; synonym: 5-fluoro-QUPIC)	0.5 mL		X		LCMSMS	30
6-APDB (formal name: 2,3-dihydro-α-methyl-6-benzofuranethanamine)	0.5 mL		X		LCMSMS	30
6-Monoacetylmorphine	2 mL, 1 mL		X	X	GCMS, LCMSMS	11, 28
7-Aminoclonazepam	1 mL		X	X	LCMSMS	25
7-Aminoflunitrazepam	1 mL		X	X	LCMSMS	25

Toxicology Laboratory Procedure	Specimen Requirement	Screen	Qual. Testing	Quant. Testing	Method(s)	Chapter(s)
AB-FUBINACA (formal name: N-[(1S)-1-(aminocarbonyl)-2-methylpropyl]-1-[(4-fluorophenyl)methyl]-1H-indazole-3-carboxamide)	0.5 mL		X		LCMSMS	30
AB-PINACA (formal name: (S)-N-(1-amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indazole-3-carboxamide)	0.5 mL		X		LCMSMS	30
Acetaminophen	1 mL		X	X	EIA, LC	8, 22
Acetone	0.1 mL, 1-2 gm tissue		X	X	HSGC	7
Acetylcodeine	2 mL		X		GCMS	9
Acetyl fentanyl	2 mL, 1 mL		X		GCMS, LCMSMS	17, 28
Acrylfentanyl	1mL		X		LCMSMS	28
ADB-FUBICA (formal name: N-(1-amino-3,3-dimethyl-1-oxobutan-2-yl)-1-(4-fluorobenzyl)-1H-indole-3-carboxamide)	0.5 mL		X		LCMSMS	30
Alcohol Panel	0.1 mL, 1-2 gm tissue	X	X	X	HSGC	7
alpha-hydroxyalprazolam	1 mL		X	X	LCMSMS	25
alpha-hydroxymidazolam	1 mL		X	X	LCMSMS	25
alpha-hydroxytriazolam	1 mL		X	X	LCMSMS	25
alpha-methylacetylfentanyl	1mL		X		LCMSMS	28
alpha-methylfentanyl	1mL		X		LCMSMS	28
alpha-PVP (alpha pyrrolidinevalerophenone)	1 mL		X	X	GCMS, LCMSMS	9, 26
Alprazolam	1 mL		X	X	LCMSMS	25
Amantadine	2 mL		X		GCMS	9
AMB-FUBINACA (formal name: N-[[1-[(4-fluorophenyl)methyl]-1H-indazol-3-yl]carbonyl]-L-valine; synonym: MMB-FUBINACA)	0.5 mL		X		LCMSMS	30
Amitriptyline	2 mL		X	X	GCMS, GC	9
Amobarbital	1 mL		X	X	GCMS	12
Amoxapine	2 mL		X	X	GCMS, GC	9
Amphetamine	1 mL		X	X	LCMSMS	26
Atomoxetine	2 mL		X		GCMS	9
Benocyclidine	2 mL		X		GCMS	9

Toxicology Laboratory Procedure	Specimen Requirement	Screen	Qual. Testing	Quant. Testing	Method(s)	Chapter(s)
Benzocaine	2 mL		X		GCMS, GC	9
Benzodioxolefentanyl	1mL		X		LCMSMS	28
Benzonatate	2 mL		X		GCMS, GC	9
Benzoylecgonine	1 mL		X	X	LCMSMS	28
Benztropine	2 mL		X	X	GCMS, GC	9
beta-Hydroxythiofentanyl	1mL		X		LCMSMS	28
Blood Alcohol Concentration (BAC)	0.1 mL		X	X	HSGC	7
Brompheniramine	2 mL		X	X	GCMS, GC	9
Bupivacaine	2 mL		X		GCMS	9
Buprenorphine	0.1 mL, 2 mL	X	X	X	EIA, LCMSMS	8, 29
Bupropion	0.2 mL		X	X	LCMSMS	26
Buspirone	2 mL		X		GCMS	9
Butabarbital	1 mL		X	X	GCMS	12
Butalbital	1 mL		X	X	GCMS	12
Butyryl fentanyl	2 mL, 1mL		X		GCMS, LCMSMS	17, 28
Caffeine	2 mL		X	X	GCMS, GC	9
Carbamazepine	0.2 mL		X	X	LCMSMS	27
Carbinoxamine	2 mL		X		GCMS, GC	9
Carbon Monoxide	5 mL	X	X	X	Chemical, S	10
Carfentanil	1mL		X		LCMSMS	28
Carisoprodol	0.2 mL		X	X	GCMS, GC	14
Cetirizine	2 mL		X		GCMS	9
Chlordiazepoxide	1 mL		X	X	LCMSMS	13, 25
Chloroform	2 mL		X		HSGC, GCMS	20
Chloroquine	2 mL		X		GCMS	9
Chlorpheniramine	2 mL		X	X	GCMS, GC	9
Chlorphentermine	2 mL		X		GCMS, GC	9
Chlorpromazine	2 mL		X	X	GCMS, GC	9
cis-3-methylfentanyl	1mL		X		LCMSMS	28
Citalopram	2 mL		X	X	GCMS, GC	9

Toxicology Laboratory Procedure	Specimen Requirement	Screen	Qual. Testing	Quant. Testing	Method(s)	Chapter(s)
Clomipramine	2 mL		X	X	GCMS, GC	9
Clonazepam	1 mL		X	X	LCMSMS	25
Clonazolam	0.5 mL		X		LCMSMS	30
Clozapine	2 mL		X	X	GCMS, GC	9
Cocaethylene	1 mL		X	X	LCMSMS	28
Cocaine	1 mL		X	X	LCMSMS	28
Codeine	2 mL, 1 mL		X	X	GCMS, GC, LCMSMS	11, 28
Crotonylfentanyl	2 mL		X		GCMS	9
Cyclopropylfentanyl	2 mL, 1mL		X		GCMS, LCMSMS	9, 28
Cyclobenzaprine	2 mL		X	X	GCMS, GC	9
Deschloroketamine	2 mL		X		GCMS	9
Desipramine	2 mL		X	X	GCMS, GC	9
Desproprionyl fentanyl	2 mL, 1mL		X		GCMS, LCMSMS	17, 28
Dextromethorphan	2 mL, 1 mL		X	X	GCMS, GC, EIA	8, 9
Diazepam	1 mL		X	X	LCMSMS	25
Dibutylone	0.5 mL		X		LCMSMS	30
Dicyclohexylamine	2 mL		X		GCMS	9
Dicyclomine	2 mL		X		GCMS, GC	9
N,N-Diethylpentylone	0.5 mL		X		LCMSMS	30
Difluoroethane	2 mL		X		HSGC, GCMS	20
Diltiazem	2 mL		X	X	GCMS, GC	9
N,N-Dimethylpentylone	0.5 mL		X		LCMSMS	30
Diphenhydramine	2 mL, 1 mL		X	X	GCMS, GC, EIA	8, 9
Dothiepin	2 mL		X		GCMS, GC	9
Doxepin	2 mL		X	X	GCMS, GC	9
Doxylamine	2 mL		X	X	GCMS, GC	9
Ethanol	0.1 mL, 1-2 gm tissue		X	X	HSGC	7
Ethyl acetate	2 mL		X		HSGC, GCMS	20
n-Ethylhexedrone	2 mL		X		GCMS	9

Toxicology Laboratory Procedure	Specimen Requirement	Screen	Qual. Testing	Quant. Testing	Method(s)	Chapter(s)
Ethylone	1 mL		X		GCMS, LCMSMS	9, 26
Ethylpentylone (synonym: N-Ethylpentylone)	2 mL, 0.5 mL		X		GCMS, LCMSMS	9, 30
Etizolam	1 mL		X		LCMSMS	25
Etomidate	2 mL		X		GCMS	9
Fentanyl	2 mL, 1 mL		X	X	GCMS, LCMSMS	17, 28
Flecainide	2 mL		X	X	GCMS, GC	9
Flubromazolam	1 mL		X		LCMSMS	25
Flubromazepam	1 mL		X		LCMSMS	25
Flunitrazepam	1 mL		X	X	LCMSMS	25
Fluoxetine	2 mL		X	X	GCMS, GC	9
Flurazepam	1 mL		X	X	LCMSMS	25
Flurazepam (N-desalkyl)	1 mL		X	X	LCMSMS	25
FUB-MDMB (formal name: N-[[1-[(4-fluorophenyl)methyl]-1H-indazol-3-yl]carbonyl]-3-methyl-L-valine; synonym: MDMB-FUBINACA)	0.5 mL		X		LCMSMS	30
Furanylfentanyl	2 mL, 1mL		X		GCMS, LCMSMS	9, 18, 28
GHB (gamma hydroxybutyric acid)	0.2 mL		X	X	GCMS	15
Glutethimide	1 mL		X	X	GCMS	12
Haloperidol	2 mL		X	X	GCMS, GC	9
Hydrocodone	2 mL, 1 mL		X	X	GCMS, LCMSMS	11, 28
Hydromorphone	2 mL, 1 mL		X	X	GCMS, LCMSMS	11, 28
Hydroxyzine	2 mL		X	X	GCMS	9, 19
Ibuprofen	2 mL		X		GCMS	9
Imipramine	2 mL		X	X	GCMS, GC	9
Isopropanol	0.1 mL, 1-2 gm tissue		X	X	HSGC	7
Ketamine	2 mL		X	X	GCMS, GC	9
Lacosamide	0.2 mL			X	LCMSMS	27
Lamotrigine	2 mL, 0.2 mL		X	X	GCMS, LCMSMS	9, 27
Levamisole/tetramisole	2 mL		X		GCMS, GC	9

Toxicology Laboratory Procedure	Specimen Requirement	Screen	Qual. Testing	Quant. Testing	Method(s)	Chapter(s)
Levetiracetam	0.2 mL		X	X	LCMSMS	27
Lidocaine	2 mL		X	X (elevated)	GCMS, GC	9
Loperamide	2 mL		X	X	GCMS, GC	9
Loratidine	2 mL		X		GCMS, GC	9
Lorazepam	1 mL		X	X	LCMSMS	25
Loxapine	2 mL		X	X	GCMS, GC	9
MAB-CHMINACA (formal name: N-[1-(aminocarbonyl)-2,2-dimethylpropyl]-1-(cyclohexylmethyl)-1H-indazole-3-carboxamide)	0.5 mL		X		LCMSMS	30
Maprotiline	2 mL		X	X	GCMS, GC	9
MDA	1 mL		X	X	LCMSMS	26
MDEA	1 mL		X	X	LCMSMS	26
MDMA	1 mL		X	X	LCMSMS	26
MDPV	1 mL		X	X	LCMSMS, GCMS	9, 26
Meclizine	2 mL		X	X	GCMS, GC	9
Meperidine	2 mL, 1 mL		X	X	GCMS, GC, LCMSMS	9, 28
Mephedrone	1 mL		X	X	LCMSMS	26
Mepivicaine	2 mL		X	X (elevated)	GCMS, GC	9
Meprobamate	0.2 mL		X	X	GCMS, GC	14
meta-fluorobutyrylfentanyl	1mL		X		LCMSMS	28
meta-fluorofentanyl	1mL		X		LCMSMS	28
meta-fluoroisobutyrylfentanyl	1mL		X		LCMSMS	28
Metaxalone	2 mL		X	X	GCMS, GC	9
Methadone	2 mL, 1 mL		X	X	GCMS, GC, LCMSMS	9, 28
Methamphetamine	1 mL		X	X	LCMSMS	26
Methanol	0.1 mL		X	X	HSGC	7
Methcathinone	1 mL		X	X	LCMSMS	26
Methedrone	1 mL		X	X	LCMSMS	26
Methiopropamine	0.5 mL		X		LCMSMS	30
Methocarbamol	2 mL		X	X	GCMS, GC	9

Toxicology Laboratory Procedure	Specimen Requirement	Screen	Qual. Testing	Quant. Testing	Method(s)	Chapter(s)
Methoxetamine	2 mL		X		GCMS	9
Methoxyacetylfentanyl	2 mL, 1mL		X		GCMS, LCMSMS	9, 28
Methoxyphenidine	0.5 mL		X		LCMSMS	30
Methylene chloride	2 mL		X		HSGC, GCMS	20
Methylone	2 mL		X	X	LCMSMS, GCMS	9, 26
Methylphenidate	2 mL		X		GCMS	9
Metoclopramide	2 mL		X		GCMS	9
Midazolam	1 mL		X	X	LCMSMS	25
Mirtazapine	2 mL		X	X	GCMS, GC	9
Mitragynine	2 mL, 0.5 mL		X		GCMS, LCMSMS	9, 30
MMB-CHMICA (formal name: methyl (1-(cyclohexylmethyl)-1H-indole-3-carbonyl)-L-valinate; synonym: AMB-CHMICA)	0.5 mL		X		LCMSMS	30
Molindone	2 mL		X		GCMS	9
Morphine	2 mL, 1 mL		X	X	GCMS, LCMSMS	11, 28
Naloxone	2 mL		X	X	LCMSMS	29
Naproxen	0.5 mL		X	X	LC, GCMS	9,
Nefazodone	2 mL		X		GCMS	9
Nicotine	2 mL		X		GCMS	9
Norbuprenorphine	2 mL		X	X	LCMSMS	29
Nordiazepam	1 mL		X	X	LCMSMS	25
Nordoxepin	2 mL		X	X	GCMS, GC	9
Norfluoxetine	2 mL		X	X	GCMS, GC	9
Norketamine	2 mL		X	X	GCMS, GC	9
Normeperidine	2 mL		X	X	GCMS, GC	9
Norpropoxyphene	2 mL		X	X	GCMS, GC	9
Nortriptyline	2 mL		X	X	GCMS, GC	9
Ocfentanil	1mL		X		LCMSMS	28
Olanzapine	2 mL		X	X	GCMS, GC	9
Ondansetron	2 mL		X		GCMS	9

Toxicology Laboratory Procedure	Specimen Requirement	Screen	Qual. Testing	Quant. Testing	Method(s)	Chapter(s)
Orphenadrine	2 mL		X	X	GCMS, GC	9
ortho-fluoroacrylfentanyl	1mL		X		LCMSMS	28
ortho-fluorobutyrylfentanyl	1mL		X		LCMSMS	28
ortho-fluorofentanyl	1mL		X		LCMSMS	28
ortho-fluoroisobutyrylfentanyl	1mL		X		LCMSMS	28
Oxazepam	1 mL		X	X	LCMSMS	25
Oxcarbazepine	0.2 mL		X	X	LCMSMS	27
Oxcarbazepine Metabolite	0.2 mL		X	X	LCMSMS	27
Oxycodone	2 mL, 1 mL		X	X	GCMS, LCMSMS	11, 28
Oxymorphone	2 mL, 1 mL		X	X	GCMS, LCMSMS	11, 28
para-fluoroacrylfentanyl	1mL		X		LCMSMS	28
para-Fluorobutyrylfentanyl	2 mL, 1mL		X		GCMS, LCMSMS	17, 28
para-fluorofentanyl	1mL		X		LCMSMS	28
para-Fluoroisobutyrylfentanyl	2 mL, 1mL		X		GCMS, LCMSMS	17, 28
Paroxetine	2 mL		X	X	GCMS	9
PB-22 (formal name: 1-pentyl-8-quinolinyl ester-1H-indole-3-carboxylic acid; synonym: QUPIC)	0.5 mL		X		LCMSMS	30
Pentazocine	2 mL		X	X	GCMS, GC	9
Pentobarbital	1 mL		X	X	GCMS	12
Pentylone	0.5 mL		X		LCMSMS	30
Phenazepam	1 mL		X	X	LCMSMS	25
Phencyclidine	2 mL		X	X	GCMS, GC	9
Pheniramine	2 mL		X		GCMS, GC	9
Phenobarbital	1 mL		X	X	GCMS	12
Phensuximide	2 mL		X		GCMS, GC	9
Phentermine	1 mL		X	X	LCMSMS	26
Phenylfentanyl	1mL		X		LCMSMS	28
Phenyltoloxamine	2 mL		X		GCMS, GC	9
Phenytoin	1 mL		X	X	LCMSMS	27
Pregabalin	0.2 mL			X	LCMSMS	27

Toxicology Laboratory Procedure	Specimen Requirement	Screen	Qual. Testing	Quant. Testing	Method(s)	Chapter(s)
Primidone	2 mL		X		GCMS	12
Procaine	2 mL		X	X (elevated)	GCMS, GC	9
Promethazine	2 mL		X	X	GCMS, GC	9
Propoxyphene	2 mL		X	X	GCMS, GC	9
Protriptyline	2 mL		X		GCMS, GC	9
Pseudoephedrine/ephedrine	1 mL		X	X	LCMSMS	26
PV8 (formal name: 1-phenyl-2-(1-pyrrolidinyl)-1-heptanone; synonym: α-PHPP)	0.5 mL		X		LCMSMS	30
Quetiapine	2 mL		X	X	GCMS	18
Quinine/quinidine	2 mL		X		GCMS	9
Salicylate	1 mL		X	X	EIA, LC	8, 22
SDB-006 (formal name: 1-pentyl-N-(phenylmethyl)-1H-indole-3-carboxamide)	0.5 mL		X		LCMSMS	30
Secobarbital	1 mL		X	X	GCMS	12
Sertraline	2 mL		X	X	GCMS, GC	9
Strychnine	2 mL		X		GCMS	9
Tapentadol	2 mL		X	X	GCMS, GC	9
Temazepam	1 mL		X	X	LCMSMS	25
Tenocyclidine (synonym: TCP)	0.5 mL		X		LCMSMS	30
Terfenadine	2 mL		X		GCMS	9
Tetrahydrocannabinol (THC)	1 mL		X	X	LCMSMS	24
Tetrahydrocannabinol, 11-hydroxy (OH-THC)	1 mL		X		LCMSMS	24
THC Carboxylic Acid (THC-COOH)	1 mL		X	X	LCMSMS	24
Tetrahydrofuranfentanyl	1mL		X		LCMSMS	28
Thiopental	1 mL		X		GCMS	12
Thioridazine	2 mL		X	X	GCMS, GC	9
Thiothixine	2 mL		X		GCMS, GC	9
Tizanidine	2mL		X		GCMS	9
Toluene	2 mL		X		HSGC, GCMS	20
Topiramate	1 mL		X	X	LCMSMS	27

Toxicology Laboratory Procedure	Specimen Requirement	Screen	Qual. Testing	Quant. Testing	Method(s)	Chapter(s)
Tramadol	2 mL, 1 mL, 0. 1 mL		X	X	EIA, GCMS, GC, LCMSMS	8, 9, 28
trans-3-methylfentanyl	1mL		X		LCMSMS	28
Trazodone	2 mL		X	X	GCMS, GC	9
Triazolam	1 mL		X	X	LCMSMS	25
Trifluroperazine	2 mL		X		GCMS, GC	9
Trihexyphenidyl	2 mL		X		GCMS, GC	9
Trimethylbenzamide	2 mL		X		GCMS, GC	9
Trimipramine	2 mL		X	X	GCMS, GC	9
U-47700	2 mL, 1mL		X		GCMS, LCMSMS	9, 28
U-49900	2 mL, 1mL		X		GCMS, LCMSMS	9, 28
Valerylfentanyl	1mL		X		LCMSMS	28
Valproic Acid	1 mL		X	X	GCMS	16
Venlafaxine	2 mL		X	X	GCMS, GC	9
Verapamil	2 mL		X	X	GCMS, GC	9
Xylene	2 mL		X		HSGC, GCMS	20
Zaleplon	1 mL		X	X	LCMSMS	25
Zolpidem	2 mL		X	X	LCMSMS, GC	25
Zonisamide	0.2 mL		X	X	LCMSMS	27
Zopiclone	1 mL		X	X	LCMSMS	25

Toxicology Testing Panels

Chapter	Testing Panel	Drugs Included	Specimen Requirement	Screen	Qual	Quant	Method(s)
7	Alcohol	Ethanol	0.1 mL, 1-2 gm tissue	X	X	X	HSGC
		Isopropanol		X	X	X	
		Methanol		X	X	X	
		Acetone		X	X	X	
11	Opiates	Morphine	2 mL		X	X	GCMS
		Codeine			X	X	
		6-Acetylmorphine			X		
		Hydrocodone			X	X	
		Hydromorphone			X	X	
		Oxycodone			X	X	
		Oxymorphone			X	X	
24	THC	Tetrahydrocannabinol	1 mL		X	X	LCMSMS
		THC Carboxylic Acid			X	X	
		OH-THC			X		
12	Barbiturate	Butalbital	1 mL		X	X	GCMS
		Phenobarbital			X	X	
		Amobarbital			X	X	
		Pentobarbital			X	X	
		Secobarbital			X	X	
17	Fentanyl	Fentanyl	2 mL		X	X	GCMS
		Acetyl Fentanyl			X		
		Butyryl Fentanyl			X		
		Furanyl Fentanyl			X		
		Desproprionyl Fentanyl			X		
		Cis-3-methyl Fentanyl			X		
		Trans-3-methyl Fentanyl			X		
		Para-Fluoroisobutyryl Fentanyl			X		
		Para-Fluorobutyryl Fentanyl			X		
26	Sympathomimetic	Amphetamine	1 mL	X	X	X	LCMSMS
		Methamphetamine		X	X	X	
		MDMA		X	X	X	
		MDA		X	X	X	

Chapter	Testing Panel	Drugs Included	Specimen Requirement	Screen	Qual	Quant	Method(s)
		MDEA		X	X	X	
		Phentermine		X	X	X	
		Pseudoephedrine/ Ephedrine		X	X	X	
		Mephedrone		X	X	X	
		Methcathinone		X	X	X	
		Methedrone		X	X	X	
		Alpha PVP		X	X	X	
		MDPV		X	X	X	
		Methylone		X	X	X	
		Ethylone		X	X		
25	Benzodiazepines	7-Aminoclonazepam	1 mL		X	X	LCMSMS
		7-Aminoflunitrazepam			X	X	
		Alpha-Hydroxyalprazolam			X	X	
		Alpha-Hydroxymidazolam			X	X	
		Alpha-Hydroxytriazolam			X	X	
		Alprazolam			X	X	
		Chlordiazepoxide			X		
		Clonazepam			X	X	
		Diazepam			X	X	
		Etizolam			X		
		Flunitrazepam			X	X	
		Flurazepam			X	X	
		Lorazepam			X	X	
		Midazolam			X	X	
		N-Desalkylflurazepam			X	X	
		Nordiazepam			X	X	
		Oxazepam			X	X	
		Phenazepam			X	X	
		Temazepam			X	X	
		Triazolam			X	X	
		Flubromazepam			X		
		Flubromazolam			X		
14	Carisoprodol	Carisoprodol	0.2 mL		X	X	GCMS, GC
		Meprobamate			X	X	
27	Antiepileptic (AED)	Gabapentin	0.2 mL	X	X	X	LCMSMS

Chapter	Testing Panel	Drugs Included	Specimen Requirement	Screen	Qual	Quant	Method(s)
		Levetiracetam		X	X	X	
		Lamotrigine		X	X	X	
		Zonisamide		X	X	X	
		Oxcarbazepine		X	X	X	
		Oxcarbazepine Metabolite		X	X	X	
		Topiramate		X	X	X	
		Carbamazepine		X	X	X	
		Phenytoin		X	X	X	
		Lacosamide		X	X	X	
		Pregabalin		X	X	X	
20	Volatiles	Difluoroethane	2 mL	X	X		HSGC, GCMS
		Ethyl Acetate		X	X		
		Chloroform		X	X		
		Methylene Chloride		X	X		
		Toluene		X	X		
		Xylene (o, m, p)		X	X		
21	Heavy Metals	Arsenic	20 mL urine	X			Chemical
		Bismuth		X			
		Antimony		X			
		Mercury		X			
8	Abused Drugs	Cocaine/ Benzoylecgonine	0.1 mL	X			EIA
		Opiates		X			
		Oxycodone		X			
		Methamphetamine/ MDMA		X			
		Phencyclidine		X			
		Methadone		X			
		Fentanyl		X			
8	DUID Drugs	Cocaine/ Benzoylecgonine	0.1 mL	X			EIA
		Opiates		X			
		Oxycodone		X			
		Methamphetamine/ MDMA		X			
		Phencyclidine		X			
		Barbiturates		X			

Chapter	Testing Panel	Drugs Included	Specimen Requirement	Screen	Qual	Quant	Method(s)
		Benzodiazepines		X			
		Carisoprodol/ Meprobamate		X			
		Fentanyl		X			
		Methadone		X			
		Cannabinoids		X			
		Zolpidem		X			
		Dextromethorphan		X			
		Diphenhydramine		X			
		Tramadol		X			
		Tricyclic Antidepressants		X			
		Buprenorphine		X			
8	Tox Drugs	Cocaine/ Benzoylecgonine	0.1 mL	X			EIA
		Opiates		X			
		Oxycodone		X			
		Methamphetamine/ MDMA		X			
		Phencyclidine		X			
		Barbiturates		X			
		Benzodiazepines		X			
		Carisoprodol/ Meprobamate		X			
		Fentanyl		X			
		Methadone		X			
		Zolpidem		X			
		Buprenorphine		X			
8	X Drugs	Amphetamine	0.1 mL	X			EIA
		Acetaminophen		X			
		Salicylate		X			
9	Acid/Neutral Drugs (Acid Screen)	Acetaminophen	2 mL	X	X		GCMS, GC
		Barbiturates		X	X		
		Caffeine		X	X		
		Carisoprodol		X	X		
		Glutethimide		X	X		
		Meprobamate		X	X		
		Metaxalone		X	X		

Chapter	Testing Panel	Drugs Included	Specimen Requirement	Screen	Qual	Quant	Method(s)
		Methocarbamol		X	X		
		Carbamazepine		X	X		
		Phenytoin		X	X		
		Levetiracetam		X	X		
		Zaleplon		X	X		
9	Alkaline Drugs (Base Screen)	Alpha PVP	2 mL	X	X		GCMS, GC
		Amantidine		X	X		
		Anthistamines		X	X		
		Antidepressants		X	X		
		Antipsychotics		X	X		
		Atomoxetine		X	X		
		Benzocaine		X	X		
		Benzodiazepines		X	X		
		Benzonatate		X	X		
		Benztropine		X	X		
		Caffeine		X	X		
		Chloroquine		X	X		
		Cocaine		X	X		
		Codeine		X	X		
		Cyclobenzapine		X	X		
		Dextromethorphan		X	X		
		Dicyclomine		X	X		
		Diltiazem		X	X		
		Etomidate		X	X		
		Fentanyl/analogs		X	X		
		Flecanide		X	X		
		Hydrocodone		X	X		
		Ketamine		X	X		
		Lamotrigine		X	X		
		Lidocaine		X	X		
		Loperaminde		X	X		
		Meclizine		X	X		
		Meperidine		X	X		
		Mepivicane		X	X		
		Methadone		X	X		
		Metoclopramide		X	X		
		Molindone		X	X		
		Nefazodone		X	X		
		Oxycodone		X	X		
		Pentazocine		X	X		
		Phencyclidine		X	X		

Chapter	Testing Panel	Drugs Included	Specimen Requirement	Screen	Qual	Quant	Method(s)
		Phensuximide		X	X		
		Procaine		X	X		
		Propoxyphene		X	X		
		Pseudoephedrine/ Ephedrine		X	X		
		Quinine/Quinidine		X	X		
		Strychine		X	X		
		Tapentadol		X	X		
		Tramadol		X	X		
		Trihexylphenidyl		X	X		
		Trimethylbenzamide		X	X		
		Verapamil		X	X		
		Zolpidem		X	X		
28	OpiCoc	Morphine	1.0 mL			X	LCMSMS
		Oxymorphone				X	
		Hydromorphone				X	
		Codeine				X	
		Oxycodone				X	
		6-Monoacetylmorphine				X	
		Hydrocodone				X	
		Benzoylecgonine				X	
		Tramadol				X	
		Cocaine				X	
		Meperidine				X	
		Acetylfentanyl				X	
		Cocaethylene				X	
		Fentanyl				X	
		Methadone				X	
28	Fentanyl Derivatives	3-fluorofentanyl	1.0mL		X		LCMSMS
		4-methoxyfentanyl			X		
		Acetylfentanyl			X		
		Acrylfentanyl			X		
		alpha-methylacetylfentanyl			X		
		alpha-methylfentanyl			X		
		Benzodioxolefentanyl			X		
		beta-hydroxythiofentanyl			X		
		Butyrylfentanyl			X		
		Carfentanil			X		
		cis-3-methylfentanyl			X		

Chapter	Testing Panel	Drugs Included	Specimen Requirement	Screen	Qual	Quant	Method(s)
		Cyclopropylfentanyl			X		
		Despropionylfentanyl			X		
		Furanylfentanyl			X		
		meta-fluorobutyrylfentanyl			X		
		meta-fluorofentanyl			X		
		meta-fluoroisobutyrylfentanyl			X		
		Methoxyacetylfentanyl			X		
		Ocfentanil			X		
		ortho-fluoroacrylfentanyl			X		
		ortho-fluorobutyrylfentanyl			X		
		ortho-fluorofentanyl			X		
		ortho-fluoroisobutyrylfentanyl			X		
		para-fluoroacrylfentanyl			X		
		para-fluorobutyrylfentanyl			X		
		para-fluorofentanyl			X		
		para-fluoroisobutyrylfentanyl			X		
		Phenylfentanyl			X		
		Tetrahydrofuranfentanyl			X		
		trans-3-methylfentanyl			X		
		U-47700			X		
		U-49900			X		
		Valerylfentanyl			X		
29	Buprenorphine	Buprenorphine	2.0 mL			X	LCMSMS
		Norbuprenorphine				X	
		Naloxone				X	
30	NPS	Methiopropamine	0.5mL		X		LCMSMS
		Dibutylone			X		
		4/5/6-MAPB			X		
		5-DBFPV			X		
		25H-NBOMe			X		
		4F/5F-AMB			X		
		3F-AMB			X		
		3-Fluorophenmetrazine			X		
		4-APDB			X		

Chapter	Testing Panel	Drugs Included	Specimen Requirement	Screen	Qual	Quant	Method(s)
		5-APDB			X		
		Pentylone/N,N-Dimethylpenylone			X		
		N-Ethylpentylone/N,N-Diethylpentylone			X		
		Tenocyclidine			X		
		4-chloro-α-PVP			X		
		3-Methoxy-PCP			X		
		Mitragynine			X		
		Methoxyphenidine			X		
		PV8			X		
		25C-NBOMe			X		
		Clonazolam			X		
		25I-NBOMe			X		
		5F-AB-PINACA			X		
		AB-FUBINACA			X		
		ADB-FUBICA			X		
		AB-PINACA			X		
		AMB-FUBINACA			X		
		4F-ADB			X		
		SDB-006			X		
		6-APDB			X		
		25I-NBOH			X		
		25B-NBOMe			X		
		25I-NBF			X		
		FUB-MDMB			X		
		PB-22			X		
		MMB-CHMICA			X		
		25I-NBMD			X		
		5F-PB-22			X		
		MAB-CHMINACA			X		

Appendix C – Return Codes

The following list contains codes that may be used on the Toxicology Summary Worksheet to indicate the disposition of evidence.

481	The evidence is being returned under separate cover.
482	The evidence is returned herewith.
483	The evidence is being retained for personal pickup.
484	The evidence will be returned via registered mail.
486	The results of other requested examinations will be reported separately.
487	The requested examination was terminated at the request of (name and title) on (date).
488	The disposition of the evidence and the results of other requested examinations are the subject of another report.
489	The evidence will be returned via United Parcel Service.
48A	The evidence is being returned to the Central Laboratory where it will be available for personal pickup.
48B	The evidence is being returned to the Northern Laboratory where it will be available for personal pickup.
48C	The evidence is being returned to the Eastern Laboratory where it will be available for personal pickup.
48D	The evidence is being returned to the Western Laboratory where it will be available for personal pickup.
48E	The evidence will be available at the Central Laboratory after you have received the results of all requested examinations.
48F	The evidence will be available at the Northern Laboratory after you have received the results of all requested examinations.
48G	The evidence will be available at the Eastern Laboratory after you have received the results of all requested examinations.
48H	The evidence will be available at the Western Laboratory after you have received the results of all requested examinations.
48I	The evidence is being returned to the Western Laboratory.
48J	The submitted evidence is available for pickup at the Northern Laboratory.
48K	The evidence is being returned to the Eastern Laboratory.
48L	The evidence is being returned to the Central Laboratory.
48M	The evidence is being returned to the Northern Laboratory.
48T	The evidence is being returned to the Office of the Chief Medical Examiner.

Appendix D – Method Abbreviations

The following list contains abbreviations that may be used on the Toxicology Summary and Toxicology DUI-DUID Summary Worksheets to indicate the method used for an analysis.

Abbreviation	Method Title
ABN or Base or A/N	Acid/Base/Neutral Drug Screen and Quantitation by GC and GCMS
BAC	Alcohols by Headspace Gas Chromatography
CO	Carboxyhemoglobin Saturation Determination
Cocaine	Cocaine, Cocaethylene, and Benzoylecgonine Quantitation and Confirmation by GC-MS
Opioid	Opioid Quantitation and Confirmation by GC-MS
Barbs	Barbiturate and Acid Drug Quantitation and Confirmation by GC and GC-MS
Chlordiaz	Chlordiazepoxide Quantitation and Confirmation by LCMSMS
Cariso	Carisoprodol and Meprobamate Quantitation and Confirmation by GC and GC-MS
GHB	GHB Quantitation and Confirmation by GC-MS
Valproic	Valproic Acid Quantitation and Confirmation by GC-MS
Fent	Fentanyl Quantitation and Confirmation by GC-MS
Quet	Quetiapine Quantitation and Confirmation by GC-MS
Hydroxy	Hydroxyzine Quantitation and Confirmation by GC-MS
Vol	Volatile Screen and Confirmation by Headspace GC and GC-MS
Metals	Heavy Metals by Reinsch Test
Acet	Acetaminophen and Salicylate Quantitation and Confirmation by HPLC
Cannabs	Cannabinoid Quantitation and Confirmation by LCMSMS
Benz	Benzodiazepines, Zolpidem, Zopiclone, and Zaleplon (Benz-ZZZ) Quantitation and Confirmation by LCMSMS
Amp or APD	Amphetamines, Phentermine, and Designer Stimulants Quantitation and Confirmation by LCMSMS
AED	Anti-Epileptic Drugs Quantitation and Confirmation by LCMSMS
OpiCoc	Opioid, Cocaine, Benzoylecgonine, Cocaethylene Quantitation and Confirmation by LCMSMS
BUP	Buprenorphine, Norbuprenorphine, and Naloxone Quantitation and Confirmation by LCMSMS
NPS	Novel Psychoactive Substances Qualtitative Screen and Confirmation using LCMSMS